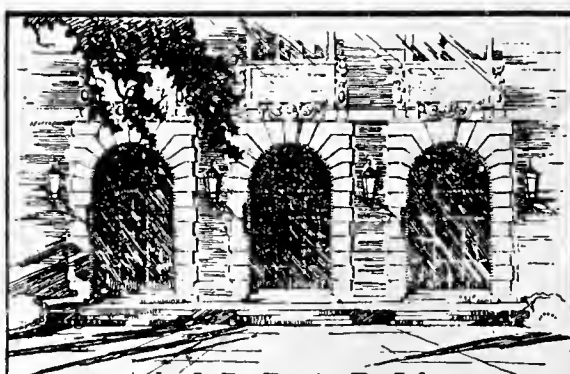




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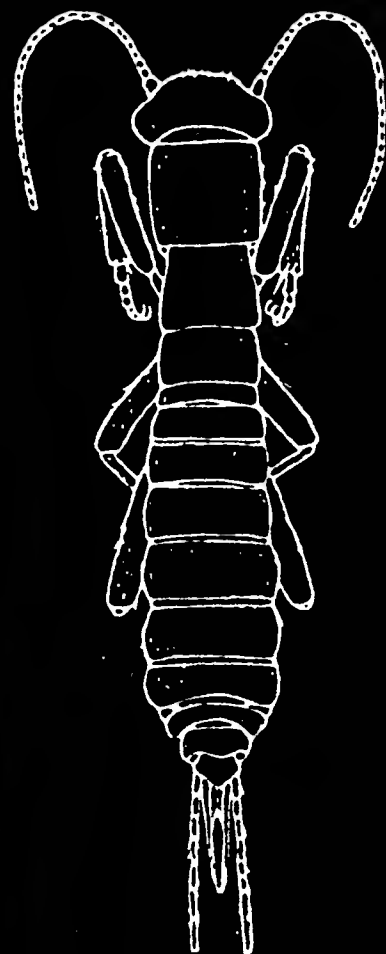
**MONTREAL, AUGUST 17-25, 1956**

**Managing Editor — Edward C. Becker**

**Published — December 1958**

**volume 3**

**AGRICULTURAL ENTOMOLOGY  
MEDICAL AND VETERINARY ENTOMOLOGY**







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# THE CONGRESS INSECT

The illustration on the cover is *Grylloblatta campodeiformis*, an insect discovered near Banff, Alberta, in 1913, by Dr. E. M. Walker, Emeritus Professor of Zoology at the University of Toronto and an Honorary Vice-President of this Congress. It is, perhaps, from the anatomical or phyletic standpoint, the most remarkable of Canadian insects. In a general sense an orthopteroid, it shows resemblances both to the Saltatoria and to the Blattaria and, together with a more recently discovered Japanese genus, ranks as an independent suborder or order.

*Grylloblatta* is adapted to cold conditions. It is found in the Rocky Mountains, near the timberline, living in decaying wood or moss among loose rock. It feeds on other insects and is nocturnal in habits.

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Philippines

By JOHN G. MATTHYSSE

Cornell University

Ithaca, N.Y.

Los Insectos Danino en el Cultivo del Arroz en Cuba

Por JOSÉ M. OSORIO R.

Habana, Cuba

# Recent Advances in Control of Temperate-Climate Fruit Flies

By B. A. PORTER

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Beltsville, Md.

## ABSTRACT

This discussion deals with advances made since 1930 in knowledge of the North American fruit fly species of the genus *Rhagoletis*. These advances may be summarized as follows:

Early in the 1930's it was discovered that certain proteins derived from yeast are important in the nutrition of *pomonella*. This information facilitated laboratory studies on the biology and control of the insect.

Effective lures and traps for various species of *Rhagoletis* have been developed since that time. The essential attractants are fermenting proteins or other materials that liberate ammonia.

Studies of their flight habits strengthened previous conclusions that the flies move about rather freely, which explains the failure of small-plot experiments.

Much attention has been given to the taxonomy of this genus. It appears that this group has a strong tendency to form segregates based on host-plant preferences. Comparatively little change has taken place in the distribution of these species.

Fumigation with ethylene dibromide and low-temperature treatments have been developed, and are available if needed to permit the movement of fruit from infested areas.

Limited advances were made in chemical control from 1930 through 1945, and more rapid ones since the appearance of the new organic insecticides. Several of these materials have been found effective against the adults, and certain of them also as soil treatments against the several stages present in or on the soil.

## INTRODUCTION

My discussion this morning will deal entirely with North American species of the genus *Rhagoletis*. For convenience these fruit flies will be discussed in three groups. The first is *Rhagoletis pomonella* (Walsh), and closely similar species; it is called the apple maggot or blueberry maggot, because of the chief fruits attacked. The second group includes *cingulata* (Loew) and associated species that attack chiefly cherry. The third is the nut-attacking group, of which the most important is *completa* Cresson.

The word "recent" appears in the general subject of this morning's program and in the titles of three of the five papers that are scheduled. Since the word has not been defined for us, each one is at liberty to make his own definition. For the purpose of my discussion I will use as a starting point the year 1930. On that date lead arsenate was firmly established as a control for *pomonella* on apple, and calcium arsenate for the same or a closely related form on semi-wild blueberries. For *completa* (the walnut husk fly), in southern California the use of cryolite was still in the experimental stage, but came into general use shortly thereafter. For the cherry fruit fly group, the most important of which is *cingulata*, lead arsenate was standard. The residue situation on apples and cherries was becoming acute, and adequate substitute insecticides were yet to be developed.

At the beginning of this period there was available considerable information about the biology of these fruit flies, but much more remained to be learned. One serious handicap in many phases of fruit fly research was the fact that there were no adequate methods of handling most of these flies in the laboratory. For the most part the flies refused to behave normally in captivity, would rarely mate, and laid practically no eggs.

Since that time numerous advances have been made in knowledge of the biology, behavior, and nutrition of the various species. Advances have also been made in control methods. Research has been carried on in at least one-third of our States. However, research on these fruit flies has not been as intensive or concentrated as recent work on certain tropical fruit flies, especially the oriental fruit fly, *Dacus dorsalis* Hend., and the Mexican fruit fly, *Anastrepha ludens* (Loew). These species are to be discussed by L. D. Christenson later in this session.

## NUTRITION

An important milestone in studies on the biology of *pomonella* was the announcement by Fluke and Allen (1931) that they had found yeast or some yeast product to be important in its nutrition. They stated that, "it seems possible that the supplying of nitrogen through the presence of dead yeast cells, which are highly protein, may be the particular aid that apple maggot adults derive from the yeast and honey water solution although enzymes or vitamins might be the essential life activating substances". This discovery was of great value to all investigators who were working with *pomonella* and related species. With proper feeding, the flies would mate and lay eggs in nearly normal fashion. Laboratory studies on their biology and on the use of insecticides then became possible.

## LURES AND TRAPS

The development of effective lures and traps for various species of *Rhagoletis* has been another important advance. During the 1920's workers in South Africa (Ripley and Hepburn, 1929) had found that the Natal fruit fly, *Pterandrus rosa* (Ksh.), was strongly attracted to fermenting lures containing "pollard", known in the United States as shorts or middlings. Some of the early work in North America with materials of this type was carried on against various fruit flies in Mexico. McPhail (1939) found that a number of protein-containing materials to which sodium hydroxide solution had been added were quite attractive to *Anastrepha striata* Schin. one of the fruit fly species with which he was working.

Following the work by McPhail, various protein materials were tested in the United States on several species of *Rhagoletis*. Boyce and Bartlett (1941) reported that several of these materials were attractive to the flies of *completa* on walnut in California. The mixture that they found most effective was a solution of glycine (2%) and sodium hydroxide (3%). About the same time Dean (1941) tested several protein materials against *pomonella* in New York State and found them promising. Similar work with *pomonella* was reported a little later in Minnesota by Benjamin and Hodson (1942).

Further studies by Hodson (1943) indicated that materials that liberate ammonia, such as household ammonia, ammonium sulfate, or ammonium acetate, were highly attractive to *pomonella* flies. As his work continued, Hodson (1948) devised a trap in which ammonium carbonate was used. This trap was an inverted pint-sized waxed cardboard container such as is used for cottage cheese. The ammonium carbonate, in dry form, was placed behind a false bottom, and the inside of the trap was covered with a sticky material to catch and hold the flies. In his work with *cingulata* in Washington, Frick (1952) developed a modification of this trap, using a quart waxed carton of the type used for frozen foods.

## FLIGHT HABITS

Early workers reached the conclusion that the flies of the genus *Rhagoletis* move around very freely, especially during the preoviposition period. In small-scale experimental plots insecticides usually seemed ineffective, whereas when applied to extensive areas some of them gave excellent control.

Field studies of flight behavior have thrown added light on this problem. Phipps and Dirks (1933) in Maine liberated marked flies in apple trees that carried a crop of fruit. Most of the flies left the trees in which they were liberated. From 4 to 12 per cent of the liberated flies were recovered, most of them at distances of 100 to 200 yards. In similar experiments in Massachusetts, Bourne *et al.* (1934) recaptured very few flies, but two were taken at distances of 728 and 568 yards from the release point. Chapman and Hammer (1934) demonstrated movement of flies within a New York orchard up to a maximum of 135 yards.

Although the experimental information on the dispersal habits of *pomonella* is still limited, it tends to support the original assumption that the flies move around freely, and that this flight habit accounts for the poor results often obtained in small-scale plots. Similar results have been obtained in Oregon with *cingulata* on cherry by Jones and Wallace (1955): They liberated about 2,000 flies tagged with radioactive phosphorus, but recovered only 39, seven of them 455 feet and one 942 feet from the point of liberation.



## TAXONOMY

Advances have also been made in the taxonomy of the genus *Rhagoletis*. However, the status of certain forms needs further clarification, as indicated by a lack of agreement among entomologists on several points.

For many years the form *zephyria* Snow was considered to be the same as *pomonella* Walsh. Many workers now consider *symphoricarpi* Curran, described in 1924, as a synonym of *zephyria*. Although it is hard to distinguish these two forms from *pomonella*, the fact that they have been taken over wide areas in the western part of the country where no form of *Rhagoletis* has ever been known to attack apple suggests that they may actually be distinct.

The status of *mendax* Curran (1932), which attacks blueberries, is also in considerable doubt. McAlister and Anderson (1935) cross-mated the apple and blueberry forms and obtained a few apparently normal larvae and puparia. However, the percentage of cross-mated flies that laid eggs was small. Similarly, some cross-breeding occurred in experiments carried on by Pickett (1937), but the proportion of successes was small. The general thought is, therefore, that *mendax* is actually the same as *pomonella*, although after breeding on blueberry for a number of generations most of the flies in the *mendax* group probably prefer it to apple.

A similar situation exists with respect to certain of the species attacking cherries. The two common ones, *cingulata* (Loew) and *fausta* (O.S.), have always been considered distinct species. The status of *indifferens* Curran (1932), described from wild cherry, has been the subject of extensive study. Jones (1945) carried on breeding studies with fly populations from wild and cultivated cherry. The two forms interbred freely, and he concluded that there were no significant differences between the two. Simkover (1953) had little difficulty in Washington in cross-breeding flies from wild cherry with flies from cultivated cherry, and produced apparently normal, fertile offspring. Later Blanc and Keifer (1955) studied the problem further and concluded that the morphological differences between the eastern and western forms of *cingulata* were great enough to justify considering them to be subspecies or perhaps even distinct species. They proposed the eastern form be referred to as *cingulata cingulata* (Loew) and the western one as *cingulata indifferens* Curran. However, they did not rule out the possibility that still other subspecific groups are present in certain areas of the Pacific States.

There seem to be no serious complications regarding the species attacking nuts. The form *completa* is considered by some to be a variety of *suavis* (Loew). However, the two forms for the most part occupy different areas, although there seems to be a little overlapping along the 100th meridian (Boyce, 1934; Benjamin and Hodson, 1949).

It is evident from the literature of the last 25 or 30 years that flies of the genus *Rhagoletis* have a strong tendency to form segregates based on host-plant preferences. After this has been going on for a long time a point may be reached where given forms for the most part restrict their attack to their favorite hosts. For instance, in some localities *pomonella* may be very common on hawthorn and totally absent from apple. Further clarification of this problem is very much needed. If, for example, the form *mendax* restricts its attack to blueberries and huckleberries and rarely transfers to apple nearby, wild blueberries would not constitute a source of infestation for apple orchards in the vicinity. The same would apply to *indifferens* in wild cherry in areas near to cultivated cherry. If there is a free interchange of fruit flies between the wild and cultivated hosts, the removal of wild cherry near commercial orchards would be justified. However, if each form maintains itself independently on the wild or on the cultivated hosts, the wild cherries could be ignored.

## DISTRIBUTION

The distribution of the various species of *Rhagoletis* in the United States has changed very little during the last 25 years. Most of them are readily transported to new localities in infested fruits, and have had ample time to occupy the areas to which they are adapted. In an intensive survey in the early 1930's in connection with the Mediterranean fruit fly campaign, *pomonella* was found (Benjamin, 1934) as far south as northern Florida, extending its known southern limit by several hundred miles. However, it does not seem to have become an economic pest in apple orchards south of the Shenandoah Valley. From time to time there have been reports of damage in Middle Atlantic areas in which the insect was

already known to be present but had not formerly been of economic importance. *R. cingulata* and related forms have found their way into the Yakima Valley and other parts of Washington where climatic conditions are such that there was originally some doubt whether they would thrive. Flies of this group have also been found in southern British Columbia. One of these forms has also appeared in California, but only at high altitudes, almost entirely on wild cherry, and in this State few commercial cherry orchards are located at such altitudes.

*R. completa*, which seems to have occurred originally only in certain plains States, reached southern California in some way, and found itself much at home in the walnut orchards there. This species seems to have extended its range recently, having been found in 1954 in Sonoma and Napa Counties in California and Yakima County, Washington, and in 1955 in Kern County, California.

Many workers have observed various natural enemies attacking one stage or another of the fruit flies. However, the general feeling is that among the species of *Rhagoletis* biological control will not reduce the population to a point satisfactory to the fruit grower. During the last few years efforts have been made to bring various fruit fly parasites into the United States in the hope that they would attack our domestic forms. Most of these parasites have been members of the genus *Opius*. During 1952 and 1953 several of the species that were available in Hawaii from the oriental fruit fly and other tropical fruit flies were introduced and colonized against *cingulata* in the Pacific Northwest. Unfortunately, these species seem to have been poorly adapted to the genus *Rhagoletis*, or to United States conditions, and there is doubt whether any of them have become established. This work has been discussed by Clausen (1956).

### INTERNAL ANATOMY

Several contributions to our knowledge of the anatomy of the digestive tract and the female reproductive system of *pomonella* have been made by Dean (1932, 1933, 1935).

### MICROORGANISMS ASSOCIATED WITH LARVAE

An interesting discovery was made by Allen and Riker (1932) that a microorganism described by them as *Phytomonas melophthora* is regularly associated with *pomonella*. This organism was proved capable of causing the rot that frequently develops following infestation by *pomonella* larvae.

### FRUIT FUMIGATION AND COLD TREATMENTS

From time to time during the last 25 years work has been done with various treatments that might be applied to fruits to permit their movement to countries or areas not infested by the fruit fly involved. Such treatments would have their greatest usefulness on very lightly infested fruit, or fruit not known to be infested but coming from areas in which it had been exposed to infestation. Heavily infested fruit would obviously not be worth treatment or shipment to distant markets.

Methyl bromide has been tested as a fumigant for apples because of actual or potential infestation by *pomonella* (Phillips *et al.* 1938; Phillips and Monro, 1939; Chapman, 1940). Under some conditions the methyl bromide injured the fruit, although it was generally possible to use this gas without causing any damage. Small-scale tests with ethylene dibromide, carried on by Richardson (1955), indicated that this fumigant is highly effective. A dosage as low as  $\frac{1}{4}$  pound per 1,000 cubic feet for two hours at 70°–77°F. gave complete mortality. In reporting on the fumigation of cherries for larvae of *cingulata*, Jones and Schuh (1953) described experiments that had been carried on some years earlier with methyl bromide. They found that heavy dosages and long exposures were required for complete control of all stages of this insect, and there was serious question whether the fruit would stand such treatments. More recently Jones (1955) carried on similar experiments with ethylene dibromide. He found that all stages of *cingulata* could be killed with a fairly light dosage of this fumigant— $\frac{1}{2}$  pound per 1,000 cubic feet—for two hours. Slight changes in flavor were detected in the fruit, although apparently these effects were not serious.

The use of low temperatures was also investigated rather thoroughly several years ago. Jones (1937) exposed pitted cherries to temperatures as low as 8°F. for periods up to

21 days. Although all larvae of *cingulata* were killed by exposure to 14° for seven days, and all in sugared cherries to 20° for seven days, he concluded that this treatment would not be practical. Chapman and Hess (1941) carried on extensive experiments with the use of cold storage to eliminate infestation by *pomonella*. Complete mortality of all stages resulted from exposure to 32° for 32 days and to 36° for 45 days.

Although these commodity treatments seem to have been used very little in the movement of fruit from areas infested with various species of *Rhagoletis* to uninfested localities, they are available if they should be needed.

### CONTROL WITH INSECTICIDES

The period 1930–1944 was marked by intensive efforts to find less objectionable and more effective insecticides for the control of various kinds of fruit flies. Such efforts were made urgently necessary by difficulties during the early 1930's with residues on fruits and vegetables. Especially objectionable in this respect was lead arsenate. However, its replacement for the control of fruit flies infesting apple and cherry proved to be a difficult task. The replacing of cryolite in the control of the walnut husk fly was likewise difficult.

For the fruit-infesting species of *Rhagoletis* various workers tried a number of arsenicals other than lead arsenate, such as calcium arsenate, manganese arsenate, zinc arsenite, and basic zinc arsenate. Other materials tested included phenothiazine, xanthone, cuprous cyanide, copper carbonate, several kinds of fixed nicotine, cryolite, derris, a mixture of lime and glue, and dusts of talc or sulfur. The nicotine compounds proved useless, but many of the others appeared to have some value. However, most of them were not sufficiently effective, or caused injury to fruit or foliage. Ground derris or cube root was fairly effective under some conditions, but was expensive because of the frequent applications needed. However, the use of this material persisted for the spraying of cherries intended for the fresh-fruit market, on which the use of arsenicals was undesirable. These materials are also still used for the control of fruit flies in cultivated blueberries, where arsenical-residue problems are difficult. With this exception, however, until the middle 1940's lead arsenate maintained its position as the best material for controlling fruit flies on apples and cherries. *R. pomonella* on semi-wild blueberries was still controlled by dusting with calcium arsenate, although this material sometimes injured the foliage. Cryolite was still the best available material for the control of the walnut husk maggot, although its effectiveness left much to be desired and seemed to be declining.

With the introduction of the new organic insecticides in the middle 1940's the picture changed rapidly. Since it is still changing, and no one insecticide has come into general use everywhere, a detailed review will not be made here. Practically all the new materials have been tested in one or more localities. Recommendations vary from State to State. Many of them suggest DDT, methoxychlor, TDE, parathion, EPN, or malathion. For *cingulata* many growers still use lead arsenate for cherries intended for canning, but on cherries for the fresh-fruit market they are using rotenone, methoxychlor, or one of the phosphorus compounds.

Another control method which has not gone far enough to discuss in any detail is the use of the poison-bait sprays, which are being employed successfully for several kinds of tropical fruit flies. In studying the nutrition of *Dacus dorsalis*, Hagen (1953) found that an important constituent in the diet of this and other tropical fruit flies is "protein hydrolysate" or related materials derived from yeast proteins. Steiner (1954), working with this and other fruit flies in Hawaii, conceived the idea of combining some of these attractive materials with certain of the quick-acting new poisons such as parathion or malathion. The protein hydrolysate attracts the flies to the spray deposits, causing them to feed there in preference to other places. With such effective and quick-acting spray mixtures it has been possible to show good results even in small-plot experiments. Mixtures of this kind are being used on a tremendous scale for the eradication of the Mediterranean fruit fly in Florida. Their adaptability in the control of the *Rhagoletis* species is yet to be demonstrated, but considerable work is under way.

Another advance has been the finding that soil treatments of isodrin, endrin, and related materials have considerable value against the several stages of *cingulata* that may be present in or on the ground. Further work is needed to establish their practical value against various species of *Rhagoletis*.

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## DISCUSSION

JOSÉ R. LABRADOR. What is the possibility of controlling fruit flies with the use of systemic insecticides (metasystox or systox)?

B. A. PORTER. Systemic insecticides have shown promise on certain tropical fruit flies, but I know of no work with systemics on the *Rhagoletis* species.



# Recent Progress in the Development of Procedures for Eradicating or Controlling Tropical Fruit Flies

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## ABSTRACT

Numerous highly toxic insecticides with demonstrated or strong potential usefulness for controlling or eradicating tropical fruit flies in many parts of the world have been formulated during the past decade. New bait sprays in which comparatively small amounts of protein hydrolysate and phosphatic insecticide are applied have been especially effective in protecting ripening or ripe fruits from attack by the oriental fruit fly (*Dacus dorsalis* Hendel). They are also effective against other species, including the Mediterranean fruit fly (*Ceratitis capitata* Weid.), the melon fly (*D. cucurbitae* Coq.), and the Mexican fruit fly (*Anastrepha ludens* (Loew)). The protein hydrolysate bait contains nutrients essential for the sexual development of the oriental fruit fly. Applications of demeton may prevent oriental fruit fly development in guavas for an entire crop. Reduction in infestations of the oriental fruit fly through male annihilation with poisoned methyl eugenol feeding stations may be an effective eradication tool when applied to entire populations. The sterile male release method also has promise for application to fruit fly problems. Control of infestations in fresh fruits and vegetables for quarantine purposes has been improved by ethylene dibromide fumigation and modifications of the vapor-heat sterilization method. Aqueous dips containing ethylene dibromide or other toxicants are also highly effective in destroying infestations in fresh fruits and vegetables, while ionizing irradiation from a cobalt 60 source has exciting possibilities because of a delayed lethal effect that prevents development of exposed immature stages beyond the pupal stage.

An extensive infestation of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) was discovered in Florida in April 1956, and pest-control, regulatory, and research agencies soon became deeply involved in another all-out eradication campaign. Little more than a year before, the same fly had invaded Costa Rica. In August 1956 a single female melon fly (*Dacus cucurbitae* Coq.) was trapped for the first time in continental United States in southern California. These events dramatically emphasized the importance of tropical fruit flies and their potential for movement in this modern age of high-volume land, sea, and air travel and commerce, despite strict quarantine precautions to prevent their spread.

Tropical fruit flies take a heavy toll of fruit and vegetables in many regions of the world. They also cause the erection of trade barriers in fresh food commodities, create difficult quarantine and regulatory problems, and may even prevent the development of desirable agricultural crops. A not inconsiderable item chargeable to these flies is the cost of control and eradication projects undertaken when they threaten previously uninfested areas. The successful eradication of the Mediterranean fruit fly in Florida in 1929-30 required approximately \$7,500,000, and losses several times as great were suffered by fruit and vegetable growers. Indications are that the campaign initiated in Florida in 1956 will require an expenditure of public funds considerably in excess of the amounts needed in 1929-30. Eradication or area-control projects concerned with the Mexican fruit fly (*Anastrepha ludens* Loew) in southern California, Texas, and northern Mexico, and with the Mediterranean fruit fly and the Queensland fruit fly (*Dacus tryoni* Frogg.) in Australia are currently imposing heavy burdens on taxpayers and on pest-control and regulatory agencies.

Impetus for the substantial recent progress in the development of more effective procedures for controlling and eradicating tropical fruit flies has come from two major sources. In common with all phases of entomology, this research has benefited greatly from discoveries in other branches of science and from the welcome array of new materials, methods, equipment, and even new approaches to insect control appearing on the scene in post-World War II years. Also contributing strongly have been the incentives provided by recent emergency fruit fly situations. It is of interest that funds in excess of \$3,000,000 have been made available for cooperative fruit fly research in Hawaii since 1948, when populations of

<sup>1</sup> Unpublished data from studies conducted by L. F. Steiner, J. W. Balock, Irving Keiser, Frank G. Hinman, Kiichi Ohinata, and other associates in the cooperative fruit fly investigations, Honolulu, T. H., J. G. Shaw, M. McPhail, James F. Cooper and W. E. Stone, U.S. Dept. of Agriculture and Mexican Fruit Fly Laboratory, Mexico City, and by Andre Myburgh of the Union of South Africa are gratefully acknowledged.

the recently discovered oriental fruit fly (*Dacus dorsalis* Hendel) reached alarming proportions. This research has been conducted by the Hawaii Agricultural Experiment Station, Territorial Board of Agriculture and Forestry, University of California Agricultural Experiment Station, U.S. Department of Agriculture, Pineapple Research Institute of Hawaii, and Hawaiian Sugar Planters' Association Experiment Station.

Foremost in importance among recent chemical-control developments have been the new organic insecticides with their enlarged possibilities for eliminating infestations. Many of these—such as DDT, methoxychlor, benzene hexachloride, dieldrin, heptachlor, and organic phosphorus compounds including parathion and malathion—are highly toxic to tropical fruit flies. Several have demonstrated their effectiveness under a variety of field conditions in Mediterranean countries, South Africa, Australia, South America, Pacific Islands, Mexico, and elsewhere, and some have been used extensively in both cover sprays and sweetened-bait sprays. This has been despite residue problems resulting from the need to protect ripe and ripening fruits and vegetables from fruit fly attack up to the point of harvest, timing difficulties, variability in performance associated with weather conditions, and economic situations not always favorable for adoption of improved control methods. A detailed review of the extensive literature reporting the results of tests with cover and sweetened-bait sprays is beyond the scope of this paper.

The rapid killing action and residual effectiveness of some of the new materials have also made it possible to get good reductions in infestation with restricted applications to vegetation harboring adult flies, as illustrated by the border-spray method developed for melon fly control in Hawaii (Holdaway *et al.* 1947; Nishida and Bess 1950; Bess and Nishida 1951; Ebeling, Nishida, and Bess 1953).

Recent experiments have indicated that control of fruit flies with systemic insecticides may also be feasible. In Hawaii, oriental fruit fly infestation in guavas has been prevented for several weeks by a heavy application of demeton to the foliage. A practical systemic treatment, if one can be found, might be useful for eliminating fly production in wild hosts and, if there are no objectional residues, for controlling fruit flies attacking food crops as well.

Control of tropical fruit flies with soil applications is another usage in which there is renewed interest because of the high toxicity, fumigant action, and residual qualities of new organic insecticides. Endrin, dieldrin, and heptachlor have shown unusual promise for use on soils. One or more of these materials has already been utilized extensively to supplement other control measures in fruit fly eradication projects in California and Florida.

The eventual role soil insecticides may play in tropical fruit fly control provides interesting speculation. If their initial high toxicity persists long enough or can be maintained fairly constant, adequate control or perhaps even eradication may be possible in areas where the soil beneath all hosts can be treated. Soil applications to individual farms or orchards subject to attack from flies coming from untreated localities probably would afford little protection. Most of the tropical fruit flies are strong fliers and readily attracted by ripe or ripening fruits and vegetables, and a soil application would be no deterrent to them. Use of soil insecticides to supplement spray programs in eradication campaigns appears to be warranted on the basis of our present knowledge. Less or greater usage may be desirable in future campaigns after experience has provided a broader basis for evaluating the self-sufficiency of a spray program and the need for supplemental soil treatments. Unfortunately, work on soil insecticides has lagged behind other aspects of tropical fruit fly investigations because of the greater promise found in cover and poisoned-bait sprays.

A highly significant chemical-control development during the last decade is the protein hydrolysate bait spray formulated by Steiner (1952, 1954, 1955, 1955a). This unusual spray exploits the classical poisoned-bait concept of fruit fly control first advocated by Malley in South Africa and Berlese in Italy shortly after the turn of the century. It also takes advantage of the rapid killing action and residual toxicity of wettable-powder formulations of organic-phosphorus compounds and the apparently insatiable appetite at least some fruit flies have for protein hydrolysates. Prerequisite for this spray was the discovery that certain protein hydrolysates contain nutritive elements essential for sexual development of adult fruit flies (Hagen and Finney 1950; Hagen 1953). When Steiner incorporated the enzymatic yeast hydrolysates in sprays with organic-phosphorus wettable powders, the flies were attracted, for short distances at least, and the poison residues elicited an immediate feeding response which even transcended ovipositional urge. With kill the result of both contact-



and stomach-poison action, adults of some fruit fly species could be eliminated with as little as 4 to 8 ounces of parathion or malathion per acre. The enzymatic yeast and soy protein hydrolysates Hagen and Finney worked with were only moderately attractive to fruit flies in olfactometer tests conducted by Gow (1954).

Strangely enough, the protein hydrolysates have not increased the effectiveness of most of the chlorinated hydrocarbon insecticides studied in Hawaii. It remains to be determined whether this is because of their comparative insolubility and the inability of the fruit flies to ingest solid particles and thus permit contact to be augmented by stomach-poison action. With openings to the pseudotracheae in the mouth parts of the Mediterranean fruit fly only 2.5 to 3.0 microns (Hanna 1947), most of the insoluble particles in conventional powder formulations would be too large for ingestion. Studies with ultra-fine organic-phosphorus and chlorinated hydrocarbon wettable-powder formulations, which the flies might ingest in toto if it is practical to produce them may indicate opportunities for even greater benefits from protein hydrolysate bait sprays.

Protein-containing wheat bran was used in a poisoned bait developed in western Australia as early as 1924 (Newman 1924), and unhydrolyzed casein was later used in a poisoned bait prepared in Egypt (Hanna 1947). These baits were not used in sprays, but in special containers or impregnated on bundles of plant stems suspended in host vegetation.

There is a growing list of protein hydrolysate materials that can be used in bait sprays. They include enzymatic or acid hydrolysates prepared from yeast, wheat, corn, soybeans, and milk or other animal proteins. Steiner and his associates found most fungicides to decrease bait-spray effectiveness markedly. Organic-phosphorus insecticides such as DDVP, Diazinon, Dipterex (Bayer 13/59), and Chlorthion were effective in the bait sprays but less so than parathion or malathion. Protein hydrolysate bait sprays containing parathion or malathion were considerably more effective for Mediterranean fruit fly, melon fly, and oriental fruit fly control in tests in Hawaii than sugar-parathion bait sprays now used extensively in South Africa (Myburgh and Stubbings 1950) and elsewhere. There is growing evidence that the protein hydrolysate bait sprays attract other fruit flies, including the walnut husk fly (*Rhagoletis completa* Cress.)<sup>2</sup> and the Mexican fruit fly (Shaw 1955).

The new protein hydrolysate bait spray was projected into national and international prominence by its adoption in recent years as the major weapon for the Mexican fruit fly eradication campaign in southern California and northern Mexico, and for the much more extensive Mediterranean fruit fly eradication campaign in Florida initiated in 1956. In the latter campaign the Florida Plant Board and the USDA Plant Pest Control Division applied several millions of gallons of this spray to more than 750,000 acres with four-motored C82, B17, and smaller aircraft. The usual rate per acre was 1 gallon of spray containing 2 pounds of malathion 25 per cent wettable powder and 1 3/4 or 2 pints of liquid protein hydrolysate bait. Greater Miami, with its many thousands of homes and buildings, automobiles, and other facilities and attributes of a modern metropolis, was included in the comprehensive coverage. Despite numerous difficult problems associated with this program, the infested area has already been greatly reduced and few fruit flies were found in the most recent surveys. Application of an agricultural spray to terrain inhabited by hundreds of thousands of people has been possible only because of the low mammalian toxicity of malathion, and the small quantities required per acre when combined with protein hydrolysate.

A method with unusual possibilities for fruit fly control and eradication has been suggested by the successful campaign against the screw-worm fly (*Callitroga hominivorax* (Cqrl.)) on the island of Curacao (Baumhover *et al.* 1955), in which the natural population was overflooded with irradiated sterile males. Required for this method are means of mass-producing the insect, a practical instrument for applying dosages of ionizing irradiation that will achieve the desired sterility without adversely affecting mating habits, and facilities for distribution or ability of treated insects to disperse themselves with a minimum of delay after release and compete on even terms with normal males. If these attributes can be combined in an irradiation dosage for fruit flies that will also inhibit ovipositional activity, objections to the method that might result from rot injury associated with fruit fly stings may be avoided. The method also requires the application of fairly constant sterile male

<sup>2</sup>M. M. Barnes, University of California Citrus Experiment Station, Report for year ending December 31, 1955, to Western Cooperative Spray Project.

pressure through repeated releases of treated flies to the natural population in successive generations until it declines below thresholds essential for species perpetuation.

In preliminary experiments with fruit flies in Hawaii (Steiner and Christenson 1956), sterility in male oriental fruit flies emerging from puparia irradiated at dosages ranging from 2,500 to 10,000 r was proportional to the dosage despite multiple mating habits of this fruit fly. The most practical dosage appeared to be from 6,700 to 8,400 r, but males regained ability to fertilize females from 24 to 44 days after treatment. Higher dosages gave complete sterility of the males but increased mortality, and the emerging males were not able to compete effectively with normal males. In small-cage tests with this fly and also the melon fly and the Mediterranean fruit fly, egg fertility in populations has been inversely proportional to the ratio of sterile to normal males. In a large cage in which facilities for unrestricted reproduction were provided and releases of sterile oriental fruit fly males were made at a ratio of 10 to 1, the population declined to a very low level within a few weeks but it was not eradicated, possibly because of the fertility recovery factor in the males. In contrast, the population in the check cage quickly achieved a level where uninhibited reproduction could no longer be permitted.

Without an adequate research basis, and without tests demonstrating its effectiveness under field conditions, the sterile-male release method could not be used in the Florida Mediterranean fruit fly campaign. Nonetheless, this technique is considered to have promise both for eradication and for area or community fruit fly control. Arrangements have been completed for construction at our Hawaiian fruit fly laboratory of a cobalt-60 instrument that will provide approximately 100,000 r per hour in a 5-inch diameter container, with enough capacity for an accelerated program of laboratory tests and for pilot eradication tests on small islands. Sites in the Marianas and other island groups in the Pacific and Atlantic oceans appear to afford excellent opportunities for critical field tests. Efficient fresh or dehydrated-carrot rearing media. (Finney 1956, Christenson *et al.* 1956) and techniques for obtaining eggs and manipulating fruit flies are available for culturing several of the tropical fruit flies. Low-cost mass-rearing methods are important to any consideration of the sterile-male release method, since many millions of flies may be required for an effective campaign.

There has also been highly significant progress in the development of lures for trapping tropical fruit flies. An investigation of methyl eugenol as an oriental fruit fly lure (Steiner 1952), in addition to furnishing an important detection tool for this fruit fly, also provided a means of annihilating the male population. This lure is so powerful and the male flies feed on it so avidly that a method with promise for eradicating incipient infestations has been suggested in which the methyl eugenol is used in combination with an insecticide such as parathion or Pyrolan on fiberboard feeding stations (Steiner and Lee 1955).

The discovery of the attractiveness of angelica seed oil to Mediterranean fruit fly males in routine screening tests in Hawaii (Steiner *et al.* 1957) only a short time before this fly was found in Florida in 1956 played an important role in the present eradication campaign. A dry-trap method increased the usefulness of this and other male lures substantially (Steiner 1957). Recent lure discoveries also include the attractiveness of various esters of cyclohexenecarboxylic acid to the Mediterranean fruit fly, and of anisyl acetone to male melon flies.

In still another study Gow (1954) found that, by culture of a bacterium identified only as *Proteus* sp. on soybean meal under aerobic conditions, a lure strongly attractive to both sexes of the three Hawaiian fruit flies could be produced. Prolonged efforts to identify the attractive constituents failed, but when the concentrated organic acids in the soybean meal culture were combined with certain ammonium compounds stronger attractants resulted. Later Steiner and K. Ohinata combined ammonium chloride with enzymatic yeast hydrolysates to form lures that now are the best attractants for both sexes of these flies. However, for the Mediterranean fruit fly they were only slightly superior to a casein-sodium hydroxide lure devised by McPhail (1939).

It is of interest that in routine screening tests in Hawaii lures attractive to male fruit flies have been encountered much more frequently than materials attractive to females. In general, the attractiveness of the best male lures has been at a substantially higher level than that of the best female lures.

Control of tropical fruit flies, or their eventual eradication, may depend upon methods for preventing further spread of infestations. A significant recent step forward was the

discovery of the effectiveness of ethylene dibromide as a fruit fly fumigant for fresh fruits and vegetables (Balock and Lindgren 1951, Balock 1951). This fumigant has solved or has shown promise for alleviating a number of difficult fruit fly quarantine problems the world over. Availability of ethylene dibromide fumigation for citrus and other crops and knowledge that normal movement to market of these commodities could go on with only minor treatment inconveniences were important factors in the generally favorable public reaction to the Mediterranean fruit fly eradication program in Florida. Essential for recommendations for the use of ethylene dibromide have been the extensive fumigation, tolerance, and residue data for fresh fruits and vegetables obtained in cooperative studies by the Hawaii and California Agricultural Experiment Stations and by the U.S. Department of Agriculture at its laboratories in Honolulu, Mexico City, and Beltsville.

Short-period aqueous dips containing ethylene dibromide and other fumigants have also been effective in Hawaiian studies. With a 20-minute heated (110°–120°F.) ethylene dibromide dip Frank G. Hinman and J. W. Balock combined a fruit fly treatment with a hot-water treatment that had been developed by the Hawaii Agricultural Experiment Station (Akamine and Arisumi 1953) to inhibit storage rots and prolong shelf life of papayas.

Comparatively low dosages of ionizing irradiation, i.e., under 20,000 r, applied to immature stages of the oriental fruit fly have prevented their development beyond the pupal stage (Balock *et al.* 1956). The possibility of a quarantine treatment for fresh fruits and vegetables based on irradiation is suggested by these results. Dosages up to 300,000 r were not immediately lethal to third-instar larvae, and egg hatch was not affected by irradiations up to 36,000 r.

Recent contributions to our knowledge of the biological control of fruit flies have been described and evaluated by Clausen (1956). Substantial reductions in oriental fruit fly infestations by introduced parasites in Hawaii (van den Bosch *et al.* 1951, Carter 1952, Clancy *et al.* 1952, Bess 1953, Christenson 1953, Clausen 1956), even though falling considerably short of the control ordinarily required in commercial fruit production, provide a striking example of benefits that may accrue from a well-balanced and coordinated program. As Clausen pointed out, the general lack of success in the biological control of fruit flies outside of Hawaii may have been due in part to lack of proper rearing and colonization programs, such as those in the oriental fruit fly campaign initiated by the Territorial Board of Agriculture and Forestry in 1947 and later expanded into a comprehensive cooperative effort in 1948, rather than to failure of the parasites to adapt themselves to the fruit fly species. Continued research on fruit fly parasites, predators, and diseases, and supporting exploration, introduction, and colonization programs are still vital needs.

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# Treatments of Various Fruits and Vegetables to Permit Their Movement Under Fruit Fly Quarantines

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## ABSTRACT

A brief review is given of the cold-storage, frozen-pack, vapor-heat, methyl bromide, and ethylene dibromide treatments applied to various fruits and vegetables imported into the United States or moved from State to State for fruit fly quarantine control. The fruit flies include the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), the melon fly, *Dacus cucurbitae* Coq., the oriental fruit fly, *Dacus dorsalis* Hendel, the Mexican fruit fly, *Anastrepha ludens* (Loew), and the West Indian fruit fly, *Anastrepha mombinpraeoptans* Sein. Cold-storage and vapor-heat methods still have considerable use, the former especially for the treatment of grapes and other fruit from South Africa and Argentina, the latter especially for Hawaiian papayas. The frozen-pack method also had had wide use in Hawaii in recent years. Methyl bromide fumigation had some use in Hawaii, but except for tomatoes it has been largely replaced by the recently developed ethylene dibromide method. This fumigation may be completed in about two hours and costs less than the low-temperature and vapor-heat methods. Dosages of 3/4 pounds or less per 1,000 cubic feet are generally used at temperatures of 70°F. or above. It may also be used at temperatures near 55°. It has a wide commodity tolerance, including citrus. Ethylene dibromide now has extensive practical use for the treatment of Hawaiian, Mexican, West Indian, and Texas fruits and vegetables and recently has been adopted, along with other methods, for use in the Mediterranean fruit fly control campaign in Florida.

Five kinds of treatments are now approved in the United States for use on imported fruits and vegetables in connection with fruit fly quarantines. Two of these treatments involve exposure to low temperatures — cold storage at about 34°F. and the frozen pack at about 0° — one is a vapor-heat treatment at 110° or above, and two make use of a fumigant—methyl bromide or ethylene dibromide. These treatments will be discussed here in the approximate order of their development and practical use.

## COLD-STORAGE TREATMENT

The cold-storage treatment consists of two steps — (1) precooling the fruit to its very center to the desired temperature near 34°F. and (2) holding it at or below that temperature for 12 to about 20 days, depending on the species of fruit fly and the treatment temperature.

Following the work of Lounsbury (1907–1908) in South Africa and Hooper (1907) in Australia, Back and Pemberton (1916) in Hawaii found the cold-storage treatment to have great promise for control of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), in apples and some other fruits.

The first large-scale use in the United States was in Florida in 1929, where citrus from areas infested with the Mediterranean fruit fly was placed in cold storage before being allowed to leave the quarantined area (PQCA 246). This procedure called for a temperature of 28° for the first five hours followed by 30° for five days; however, some injury to citrus occurred and the treatment was later changed to 15 days at 30°–31° (PCQA 271).

The cold-storage method was later adopted for the Mediterranean fruit fly in imported grapes, with the same schedule (BPQ 362) or 12 days at 34° or below (BEPQ 417), and also for the Mexican fruit fly, *Anastrepha ludens* (Loew), present in citrus. In the latter case longer periods of treatment were required — 18 days at 33° or 22 days at 35° (Baker *et al.*, 1944). It was also adopted for treating Puerto Rican citrus for *Anastrepha suspensa* (Loew), the period being 15 days at 34° or below (BEPQ 505).

More recently the treatment was tested in Hawaii by J. W. Balock and T. Kozuma against the oriental fruit fly, *Dacus dorsalis* Hendel. At 37° an exposure of 12 days was sufficient for complete kill, indicating this fly to be slightly more susceptible than the Mediterranean fruit fly. The melon fly, *Dacus cucurbitae* Coq., required ten days at 37°, indicating

<sup>1</sup> E. P. Reagan and G. F. Callaghan of the Plant Quarantine Branch furnished information on recent practical usage of some of the treatments.

it to be even more susceptible. However, the low-temperature storage threshold for most of these tropical or subtropical Hawaiian fruits and vegetables is above 45°, which precludes the use of cold storage there, with possibly a few exceptions (Christenson, 1953).

This treatment was originally applied only in approved cold-storage plants which had adequate insulation, cooling capacity, and uniform forced-air circulation. The last requirement is very important in insuring thorough applications. Following the studies of Nel (1936) and L. A. Hawkins and G. G. Becker (unpublished reports) in 1937 this treatment was approved for vinifera grapes and other fruit on shipboard during the voyage from the Mediterranean fruit fly infested areas of South Africa to the United States (BEPQ 464). More recently (1949, BEPQ 583) the intransit cold treatment was also approved for use on similar fruit from areas in Argentina infested with *Anastrepha* spp. and other fruit flies.

The cold treatment of fruit would generally be a rather expensive method of killing fruit flies. But because imported fruit must be kept under refrigeration to prevent spoilage, its use as a quarantine treatment during the voyage has proved to be very practical. Since most modern refrigerated ships have adequate refrigeration to kill the flies, usually it is only necessary to control the temperatures more carefully by forced-air circulation and the use of temperature recorders.

Because of the uniform precooling and temperature regulation during the voyage, the fruit generally arrives in excellent condition. Hundreds of tons of fresh pears, grapes, apples, and other fruit from Argentina and South Africa are treated in this way each year. Considerable imported fruit that will withstand long cold storage, such as apples and pears, is treated in cold-storage warehouses on arrival at a United States northern port. The efficiency of the treatment has often been demonstrated by inspection of the treated fruit (Richardson, 1952a).

The cold-storage treatment is still one of the most-used methods of fruit fly quarantine control. Very little change has been made in the original procedure. Thus far it has been approved only for fruits, but there seems to be no reason why vegetables can not be treated in this way provided they are tolerant to the cold storage required to kill the species present.

#### FROZEN-PACK TREATMENT

The frozen-pack treatment consists of initial freezing at sub-zero Fahrenheit temperatures and subsequent storage at not higher than 0°, with a storage tolerance of plus 20°F. It differs from all other accepted methods in that the fruits or vegetables must be used very soon after the treatment temperatures are relaxed. It was first approved in the United States in 1931 for fruits from various countries (PQCA 311, Mason and McBride, 1934, and BEPQ 462) and later extended to vegetables (BEPQ 587). It is apparently effective against most all fruit flies and also against some other insects (McBride and Mason, 1934), but it is not authorized for use on commodities subject to pests that may not be destroyed by freezing. During recent years it has been used for the treatment of Hawaiian pineapple and also some guava and passion fruit preparations (Balock, 1956, unpublished).

#### VAPOR-HEAT TREATMENT

In the vapor-heat treatment the commodity is heated to 110°F. by saturated water vapor which condenses on the fruit or vegetables and gives up its latent heat. This latent heat is essential in raising the pulp temperatures evenly and quickly so as to prevent damage to the commodity. In application the saturated vapor is accompanied by a fine water mist and air under forced circulation. The pulp temperature is held at 110° for six to eight hours in order to kill various fruit flies (Baker, 1952).

Vapor heat was first used on a large scale in Florida in 1929 for control of the Mediterranean fruit fly in citrus. An eight-hour warm-up was followed by another eight-hour holding period (PQCA 252). It was later applied for quarantine control of Mexican fruit fly in citrus from Texas (Hawkins, 1932). For this fruit fly the holding period was reduced to six hours (BEPQ 472). Later an alternate schedule consisting of a six-hour warm-up with a rapid increase in temperature during the first two hours and a four-hour holding period at 110° was also allowed (BEPQ 575 and 542). Baker (1939) discussed the basis for determining the various specifications for both vapor-heat and cold-storage treatments for fruit fly quarantine control.

Vapor heat was later approved for the treatment of Mexican citrus and Manila mangoes for control of the Mexican fruit fly (Balock and Starr, 1945). It was also applied to Hawaiian fruits and vegetables for the Mediterranean fruit fly and the melon fly.

The oriental fruit fly, found in Hawaii in 1946, apparently proved more resistant to vapor heat, for it was necessary to extend the holding period of 8-3/4 hours (BEPQ 481). The following Hawaiian fruits and vegetables have been treated successfully: papayas, bell peppers, pineapples, Italian squash, and tomatoes (Balock, 1951). In addition a so-called "quick run-up" treatment to a minimum pulp temperature of 117° was adopted (PQ 481), which shortened the processing time considerably (Christenson *et al.*, 1956). In these more severe treatments stress was laid on cooling the fruits and vegetables immediately after the exposure. However occasional injury occurred, even where the procedure was carefully controlled. This injury has been attributed to season, rainfall, soil, varietal differences, and other factors (Balock, 1951; Pratt *et al.*, 1953).

Bananas, avocados, string beans, cucumbers, and several other crops will not tolerate vapor heat. In addition, in California the schedule specified for oriental fruit fly was sufficiently injurious to citrus (Claypool and Vines, 1953) and avocados (Sinclair and Lindgren, 1955) to preclude its use for these fruits. The treatment also gave a definite off-flavor to most types of California citrus.

In Hawaii vapor heat is still the method of choice for papayas, since storage rots that affect shelf life are inhibited by the heat (Christenson *et al.*, 1956). Considerable Mexican citrus has also been treated in recent years, with the shorter exposure time that is effective against the Mexican fruit fly.

### METHYL BROMIDE FUMIGATION

Methyl bromide gas is applied inside a tight metal-lined fumigation chamber provided with forced circulation of the air-fumigant mixture. It was first approved in 1940 for treatment of Hawaiian fruits and vegetables for Mediterranean and melon fruit fly control (BEPQ 510). A dosage of two pounds per 1,000 cubic feet was specified, with a three and one-half-hour exposure at 80°F. or above at normal atmospheric pressure. The treatment was applied successfully to tomatoes, bell peppers, and zucchini squash. Later it was approved, at the same dosage but with a six hour exposure, for use against the oriental fruit fly in pineapple (BEPQ 589). However, for other commodities infested with this fly a dosage of two pounds for four hours at 70° was tentatively adopted (Balock, 1951; Pratt *et al.*, 1953), since this species is not much different from the Mediterranean fruit fly in resistance to the gas.

Occasional injury to some commodities developed, even though the treatment conditions were well controlled. This injury was attributed to season, soil and varietal differences, and other causes (Balock, 1951; Pratt *et al.*, 1953). Several quarantined commodities, such as bananas, citrus, avocados, and cucumbers, will not tolerate the methyl bromide treatment (Armitage and Steinweder, 1946; Balock, 1951; Lindgren and Sinclair, 1951; Sinclair and Lindgren, 1953). Extensive tests on California vegetables by Pratt *et al.* (1953) indicate that five varieties of winter squash, three of melon, and four of tomatoes tolerate the treatment, but that artichokes, broccoli, cantaloups, chili peppers, eggplant, string beans, four varieties of summer squash, and late-fall-maturing tomatoes do not. Generally the least mature tomatoes show the most sensitivity, since the most striking injury symptom is a delay in ripening (Morris and Claypool, 1942; Pratt *et al.*, 1953). According to Jones (1940), tomatoes in the pink stage should be placed in cool storage at 55°–60°F. as soon as fumigated, but fumigated green tomatoes should first be allowed to ripen to the pink stage. Claypool and Vines (1953) reported that most California deciduous fruits were tolerant to this treatment for two hours but the regular four-hour schedule often has little margin of safety.

There is no question that the methyl bromide schedule required for control of these fruit flies is rather heavy and on the borderline of tolerance for a substantial number of the quarantined or other commodities. Methyl bromide was used as a fumigant in Hawaii for several years, but, except for tomatoes, it has been largely replaced by ethylene dibromide.

### ETHYLENE DIBROMIDE FUMIGATION

Ethylene dibromide as a fumigant was developed through the research of Balock (1951) and Balock and Lindgren (1951), who found that this gas was much more toxic than methyl

bromide to the oriental fruit fly. Mediterranean and melon fruit flies were also found very susceptible to this gas, though the former species is slightly less so than the oriental fruit fly (Christenson *et al.*, 1956).

This method was soon used for treating Hawaiian fruits and vegetables grown in areas quarantined for the three fruit flies just mentioned (BEPQ 592). A dosage of 1/2 pound per 1,000 cubic feet was specified, with an exposure period of two hours at 70°F. or above under normal atmospheric pressure. Since ethylene dibromide is of low volatility at room temperature, an electric hot plate or similar device is used to vaporize the liquid inside the fumigation chamber. Because the gas is over six times as heavy as air, forced circulation must be used to distribute it. The treatment has been successfully applied to Hawaiian bitter melons, Cavendish bananas, cucumbers, papayas, pineapples, string beans, and zucchini squash in open, unlined containers. Packaged papayas and pineapples were treated by a special schedule with extra dosage or exposure.

The shorter treatment time, lower dosage, and generally better and wider commodity tolerance are important advantages of this fumigant. Ethylene dibromide has also been found very effective against the West Indian fruit fly, *Anastrepha mombinpraeoptans* Sein., in mangoes and against *Anastrepha suspensa* (Loew) in almendras (tropical almonds) from Puerto Rico (Richardson, 1952b). It has been used on a practical scale at a similar dosage for the treatment of Puerto Rican and West Indian mangoes.

In extensive studies the following California fruits and vegetables have been found tolerant to the ethylene dibromide treatment: citrus and avocados (Lindgren and Sinclair, 1951; Sinclair and Lindgren, 1953); apples, apricots, boysenberries, cherries, figs, grapes, nectarines, peaches, pears, plums, and strawberries (Claypool and Vines, 1953); beans (snap and lima), broccoli, cucumbers, six varieties of melons, bell peppers, four varieties of summer squash, and five varieties of winter squash (Pratt *et al.*, 1953). The treatment caused injury to artichokes, cauliflower, eggplant, Crenshaw melons, chili peppers, four varieties of tomatoes (Pratt *et al.*, 1953), and persimmons (Claypool and Vines, 1953).

Claypool and Vines found some temporary off-flavor where small amounts of a commodity were fumigated in large chambers, but no differences in some large-scale fumigations; they concluded that the margin of safety is sufficient for most deciduous fruits and that dosages higher than 3/4 pound per 1,000 cubic feet for two hours could probably be used without danger. Lindgren and Sinclair (1951) and Sinclair and Lindgren (1953) indicate that this dosage and exposure are satisfactory for California citrus and avocados. According to Christenson *et al.* (1956), ethylene dibromide up to 1-1/2 pounds for four hours at 70° did not give complete kill of the oriental fruit fly in Hawaiian avocados. Vacuum fumigation showed promise, but further work is needed. Since avocados varied greatly in their tolerance to these higher dosages, each variety should be tested separately.

Shaw and Lopez (1954) found ethylene dibromide fumigation effective against the Mexican fruit fly in Manila mangoes, and it has since been adopted for use on this commodity at a dosage of one pound per 1,000 cubic feet for two hours at 77°F. or above. This fumigant has also been shown to be very effective against the apple maggot, *Rhagoletis pomonella* (Walsh), (Richardson, 1955) and the cherry fruit fly, *Rhagoletis cingulata* (Loew), (Jones, 1955). Recently a dosage of ten ounces per 1,000 cubic feet at 70°–76°, or eight ounces at 77° or above, with a two-hour exposure, under normal atmospheric pressure, was adopted following the work of McPhail (unpublished reports) for the treatment of citrus grown in the Mexican fruit fly quarantined areas of Texas (PPC 575). McPhail also reported that a recirculation system for the gas-air mixture makes possible the use of lower dosages with resultant increase in citrus tolerance.

Thus far all practical fumigations with ethylene dibromide have been at 70°F. or above. In some cases it would be an advantage to be able to use lower temperatures. Preliminary tests with the West Indian fruit fly (Richardson and Roth, 1956) indicated that ethylene dibromide had considerable efficiency at near 55° and that a minimum dosage near 3/4 pound per 1,000 cubic feet for two hours would be required in a 2,600 cubic foot chamber with a 40 to 45 per cent load of mangoes, packed in regular commercial crates. Considerable gas was apparently sorbed by the fruit, crates, and packing in the practical-scale cool-temperature fumigation; this sorption being similar to if not more so than that noted by Claypool and Vines (1953), and Sinclair and Lindgren (1953) in fumigations at temperatures of 70° or



above. Balock and co-workers (unpublished) also found it effective at lower temperatures against the oriental fruit fly.

Although ethylene dibromide fumigation is not the answer to all fruit fly problems, as pointed out by Christenson *et al.* (1956), it has helped greatly by making possible the treatment of various fruits and vegetables for which no treatment was previously available. Its low cost is also an advantage. Following its adoption in Hawaii, fruit and vegetable shipments, mostly fresh papayas and pineapples, expanded from a few thousand pounds in 1948 to about three million pounds in 1952 (Christenson, 1953). According to Lindgren and Sinclair (1953), 80 per cent of the Hawaiian shipments in 1952 were given the ethylene dibromide fumigation, ten per cent were given methyl bromide, and ten per cent vapor heat. The ethylene dibromide usage has since declined some because of removal of treatment requirements from pineapple and increasing preference for vapor heat for treating papaya.

Ethylene dibromide fumigation has recently been adopted for use along with other measures in the Mediterranean fruit fly eradication campaign in Florida started in May, 1956. The same dosage schedule as used for the Mexican fruit fly on citrus in Texas is being generally used.

The matter of bromide residues in treated fruits and vegetables is being investigated. Continued use of this fumigant will depend on the tolerances set by the United States Food and Drug Administration (FDA 1956).

### ETHYLENE CHLOROBROMIDE FUMIGATION

Ethylene chlorobromide has also been found to be very toxic as a fumigant for the oriental or other fruit flies (Balock and Lindgren, 1951; Christenson, 1953). Hinman (1954) rated it as the most promising next to ethylene dibromide of a total of 230 compounds tested, but, in laboratory tests about three to four times as much was required to kill 95 per cent of the naked eggs or larvae of the oriental fruit fly. From a practical standpoint this difference in efficiency may not be great, as ethylene chlorobromide has a somewhat higher vapor pressure and there does not appear to be so much loss by sorption to the fruit load, packing, and other exposed surfaces. Lindgren and Sinclair (1951), Sinclair and Lindgren (1953), Claypool and Vines (1953), and Pratt *et al.* (1953) also have found ethylene chlorobromide to have much promise from the commodity-tolerance standpoint. This fumigant may be considered as a possible future treatment, but it has not yet been officially adopted for any fruit fly quarantine control.

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## APPENDIX

### U.S. Department of Agriculture Quarantines and Administrative Instructions

#### Plant Quarantine and Control Administration (PQCA):

- 246. Sept. 20, 1929. Sterilization of citrus fruits under Mediterranean fruit fly regulations.
- 252. Oct. 23, 1929. Sterilization of grapefruit by use of heat under Mediterranean fruit fly regulations.
- 271. Mar. 4, 1930. Additional method of sterilizing Florida citrus fruits by refrigeration authorized.
- 311. May 8, 1931. The importation under permit of frozen pack fruits authorized.

#### Bureau of Plant Quarantine (BPQ):

- 362. May 1, 1934. Sterilization of imported vinifera grapes by refrigeration.

#### Bureau of Entomology and Plant Quarantine (BEPQ):

- 417. Nov. 9, 1936. Sterilization of imported vinifera grapes by refrigeration.
- 462. Sept. 15, 1937. Restrictions affecting the importation and interstate movement of frozen-pack fruits.
- 464. Sept. 15, 1937. Importation of vinifera grapes and certain other deciduous fruits subject to intransit sterilization authorized.
- 472. Apr. 8, 1938. Sterilization of grapefruit and oranges by heat under the Mexican fruitfly quarantine.
- 481. Oct. 19, 1949. Vapor heat treatment approved for certain fruits and vegetables under Hawaiian fruit and vegetable quarantine No. 13.
- 505. Jan. 4, 1940. The shipment of oranges and grapefruit from Puerto Rico to the mainland subject to treatment under supervision is authorized.
- 510. Aug. 1, 1940. Amending authorization of the shipment of fruits and vegetables from Hawaii to the mainland subject to fumigation with methyl bromide under supervision.
- 542. Rev. Nov. 20, 1952. Amendment of administrative instructions prescribing method of treatment of certain fruits from Mexico. Rev. Dec. 11, 1953. Modification of administrative instructions prescribing method of treatment of oranges, grapefruit, tangerines, and Manila mangoes from Mexico.
- 575. Feb. 1, 1949. Administrative instructions prescribing methods of treatment of grapefruit and oranges under Mexican fruitfly quarantine.
- 583. Nov. 17, 1949. Administrative instructions for cold treatments of imported vinifera grapes and certain other fruits.
- 587. Mar. 9, 1950. Administrative instructions authorizing the importation from foreign countries of frozen fruits and vegetables.
- 589. May 31, 1950. Methyl bromide fumigation approved as a condition for certification of pineapples under Hawaiian fruit and vegetable quarantine No. 13.

592. Apr. 20, 1951. Ethylene dibromide fumigation approved as a condition for certification of certain fruits and vegetables under Hawaiian fruit and vegetable quarantine No. 13.

Plant Quarantine Branch (PQ):

481. Amended Sept. 24, 1954. Amendment of administrative instructions prescribing methods of vapor heat treatment of certain fruits and vegetables from Hawaii.

Plant Pest Control Branch (PPC):

575. Rev. Dec. 23, 1955. Revised administrative instructions prescribing methods of treatment of citrus fruits under Mexican fruit fly quarantine.

U.S. Food and Drug Administration (FDA):

21. CFR, Part 120. Aug. 6, 1956. Notice of proposal to establish tolerances for residues of inorganic bromides in or on certain raw agricultural commodities after fumigation with ethylene dibromide.



# Honeydew as an Adult Fruit Fly Diet Affecting Reproduction

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## ABSTRACT

When adult *Dacus dorsalis* Hendel fed on the honeydew excreted by *Planococcus citri* (Risso), the resulting fecundity and fertility was comparable to that induced by a formulated purified diet. Since gross nutritional requirements of the adult *D. dorsalis* had been determined experimentally, it is suggested that honeydew contains carbohydrates, hydrolyzed protein, minerals and certain B-group vitamins. Furthermore, diets containing enzymatic protein hydrolyzates of either yeast or soy also permitted egg production to the degree obtained from a honeydew diet. The Mexican fruit fly, *Anastrepha ludens* (Loew), also deposited eggs when exposed to honeydew while no egg production occurred when the adults of this species were exposed to a carbohydrate alone. A search of the literature tends to support the hypothesis that honeydew excreted by homopterous insects is complex, and that various tephritid species probably seek honeydew as a natural food.

The nutritional requirements for reproduction in the Tephritidae differ somewhat between species. These differences are primarily a reflection of the quantity and quality of metabolites transferred from the immature stages to the adult. However, in the final analysis most of the tephritid species that have been studied must obtain, as adults, nearly all the nutrients required for high egg production (fecundity) and good egg hatch (fertility) from extrinsic sources (Hagen, 1952).

The species of *Rhagoletis* such as *completa* Cresson, *pomonella* (Walsh) and *cingulata* (Loew) apparently are able to produce some eggs on a carbohydrate and water diet alone (Boyce, 1934; Dean, 1938; Kamal, 1954, respectively). *Ceratitis capitata* (Wiedemann) is another species which can produce a few eggs on this simple type of diet (Hanna, 1947). However, Marlowe (1945) found that *Dacus cucurbitae* Coquillett does not produce any eggs on a carbohydrate diet alone, and *Dacus dorsalis* Hendel showed no apparent ovi-genesis if fed only carbohydrate and water (Hagen, 1952). *Anastrepha ludens* (Loew) is another species which belongs to this same category.

Fluke and Allen (1931) working with *R. pomonella*, and other above cited authors, found when brewers' yeast was added to the carbohydrate and water diet that fecundity and longevity were increased. The main conclusion was that it was the protein in the yeast that increased egg production. However, besides providing protein, yeast also contains vitamins and minerals which naturally are important.

It was shown in *D. dorsalis*, *D. cucurbitae* and *C. capitata* by using chemically defined diets that the adults required a carbohydrate, an amino acid mixture, a salt mixture, and a mixture of B vitamins before high fecundity and fertility could be obtained (Hagen, 1952, 1953).

The effects of omitting one or more of these major nutritional categories from the purified diet on *D. dorsalis* fecundity and fertility during the first 30 days are shown in Fig. 1.

The data shown in Fig. 1 indicate that before high production of fertile eggs could be obtained the diet had to be "complete". Essentially the same responses were obtained from *D. cucurbitae* and *C. capitata* to the experimental diets used by *D. dorsalis* (Hagen, 1952).

Looking closer at the requirements of these fruit flies it was found that a carbohydrate had to be present for energy. In this connection fructose, glucose, and sucrose were nearly equal in sustaining life. Dextrin was poor. The sugar concentration most frequently used was 38 per cent in liquid diets and 70 per cent in solid diets.

In an appraisal of protein requirements, protein nitrogen supplied in the form of free amino acids was superior to unhydrolyzed protein. If dried brewers' yeast mixed with a carbohydrate is fed to *D. dorsalis*, the average preoviposition period is 21 days with a mean daily egg production of 3.5. eggs. But when brewers' yeast is provided in an enzymatically hydrolyzed protein form and mixed with carbohydrate, the preoviposition period

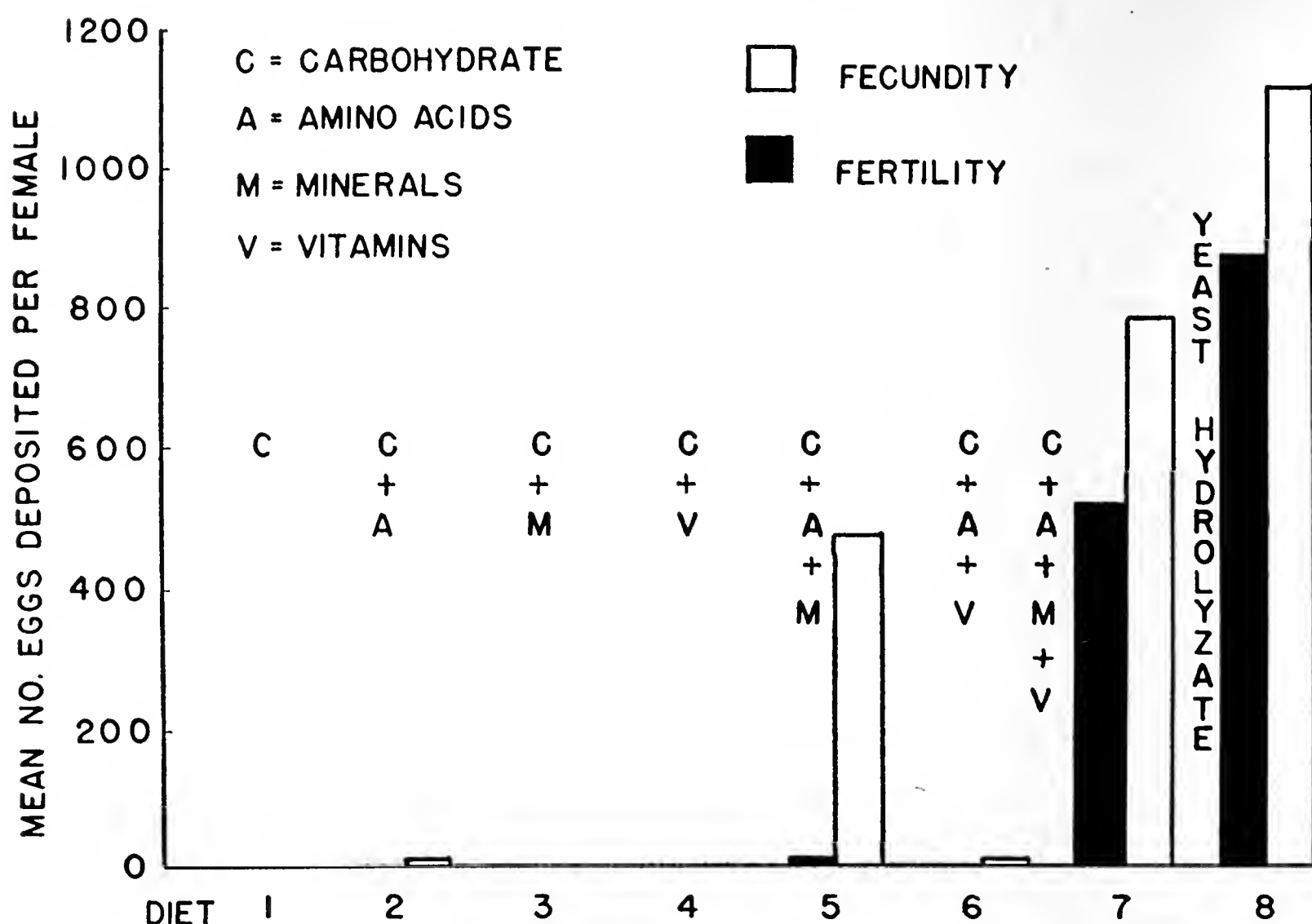


Fig. 1. Effect of major nutritional deficiencies upon the fecundity and fertility of *Dacus dorsalis*.

is one-half as long and mean daily egg production is nine times greater. Partially hydrolyzed protein like proteose-peptone mixed with yeast and a carbohydrate is intermediate in its stimulation of egg production, and Dean (1938) found that proteose-peptone was superior to brewers' yeast when fed to *R. pomonella*. In 1952, the author found the protein concentration to influence fecundity in *D. dorsalis*. The higher the concentration of amino acid mixture up to 28 per cent dry weight the greater the egg production. No significant difference in fecundity was found between feeding 19 amino acids and 13 amino acids. The latter mixture contained all the so-called essential amino acids plus glutamic acid.

The minerals present in U.S.P. salt mixture No. 13 or Nutritional Biochemicals Corp. salt mixture No. 2 contained the minerals which permitted high fecundity and fertility. Without this salt mixture the amino acids apparently could not be utilized for no eggs were produced and longevity was reduced. The concentration of the salt mixture used was 1.4 per cent in fluid diets or 2.6 per cent in solid diets.

Of the nine B vitamins tested, the omission of thiamin, nicotinic acid, folic acid, or choline chloride caused a significant decrease in fecundity or fertility.

Not only do the females require this complex of nutrients for effective fecundity and fertility but the males also must ingest a complex diet before they are able to copulate, for on a carbohydrate alone they will not mate (Hagen, 1952). Thus both the adult males and females of at least *D. dorsalis*, *D. cucurbitae* and *C. capitata* must obtain a complex diet before effective reproduction is permitted.

Where in nature can the tephritids find free amino acids preserved in high concentrations of carbohydrates? I believe that honeydew constitutes the main source of these egg-producing nutrients. Many entomologists have observed fruit flies feeding upon honeydew in the field.

Apparently one of the first records of tephritids feeding upon honeydew was Silvestri's (1914) observation on *Dacus oleae* (Gmel.) in Italy. This species not only fed upon exudations naturally occurring from olives or issuing from punctures made by the ovipositor of the fly, but also fed on honeydews excreted by Lecaniine scales. Back and Pemberton (1917) reported that *D. cucurbitae* fed upon honeydew excretions of various homopterous insects as well as upon other foods in Hawaii. Severin (1917) observed adults of *Epochra canadensis* (Loew) lapping up honeydew excreted by aphids on currant leaves.



Boyce (1934) stated that honeydew resulting from walnut aphid, *Chromaphis juglandicola* (Kltb.), infestations undoubtedly constitutes a portion of the food of *R. completa*. Boyce concluded that quantities of honeydew and spores of fungi are available for food through the period of fly activity; he also mentioned other food sources. According to Skwarra in Baker *et al.* (1944) *A. ludens* appeared to be repelled by aphid honeydew. *Dacus dorsalis* was observed feeding upon honeydew excreted by the green scale, *Coccus viridis* (Green) on guava. They also fed on honeydews from various aphids on citrus trees in Hawaii (Hagen, 1952). Steiner (1955) found that honeydew excreted by green scale on guava may compete with bait sprays in attractiveness and reduce the effectiveness of these sprays as much as 45 per cent. In India, Batra (1954) observed *Dacus diversus* (Coq.) feeding on the honeydew excreted by *Aphis gossypii* Glover.

A few experiments have been conducted in the laboratory to determine if fruit flies could live if only honeydew was provided. McBride (1935) found that in absence of suitable fruits adult *C. capitata* are capable of existing for long periods on other foods. He kept adults alive for more than three months on caged plants infested with scales, aphids and mealybugs when the only food available was honeydew excretions from the insects on the plants. In another test, Middlekauff (1941) fed *R. pomonella* honeydew excreted by the rose aphid, *Macrosiphum rosae* (L), to determine how long the apple maggot adults could live. He found the average survival period of the females was 21.6 days and the males 29.8 days. The controls fed yeast and honey survived an average of 40 days. Dr. Keizo Yasumatsu reported personally that he kept *Tetradacus tsuneonis* Miyake in Japan alive longer on honeydews excreted by a citrus aphid and *Ceroplastes rubens* Mask. than on various carbohydrate solutions that he tested.

Two preliminary experiments have been conducted to determine if fruit flies could produce eggs if exposed only to honeydew. In Hawaii, during 1951, the honeydew used was that excreted by *Planococcus citri* (Risso). The honeydew was obtained by placing heavy wax paper beneath trays supporting sprouting potatoes infested with the citrus mealybug. Sections of the wax paper where dense droplets of honeydew had fallen were placed in small cages with *D. dorsalis* adults. Along with this diet enzymatic protein hydrolyzates of yeast and soy with carbohydrates were run simultaneously as well as a chemically defined diet. Only five females and five males were used with each diet and run for 30 days, including the preoviposition period. The results from this experiment are shown in Table I.

TABLE I. Comparative Influence of *Planococcus citri* Honeydew and Artificial Diets upon the Preoviposition Period, Fecundity, and Fertility of *Dacus dorsalis* over First 30 Days. Temp. 80°F.  $\pm$  5°, Rel. Humidity 55%  $\pm$  10.

Diet	Minimum preovi- position period days	Mean No. eggs per ♀ per day	Mean No. eggs deposited per ♀	Percent hatch
Honeydew	10	24.7	864.5	84
Pure chemical diet*	10	25.6	683	64
Protein hydrolyzate of soy**	10	27.8	838	70
Protein hydrolyzate of yeast***	10	36.9	1169	68

\*Details on composition (Hagen, 1952).  
\*\*Enzymatic (trypsin) protein hydrolyzate of soy from Nutritional Biochemicals Corp.  
\*\*\*Enzymatic protein hydrolyzate of yeast from Marvin R. Thompson, Inc.

The data shown in Table I indicate that the preoviposition period was similar between all the diets tested. The differences in the numbers of eggs deposited were not significant by analysis of variance when comparing the means of daily egg production. These means were based on the total number of eggs deposited by each series of flies divided by the total number of days the females lived during the 30 day test period. Since there were no significant differences among the means, the honeydew that was tested can be compared in composition with the pure chemical diet as well as with the enzymatic protein hydrolyzates.



Since it has been more or less determined what dietary factors must be obtained from extrinsic sources by *D. dorsalis* for fecundity and fertility, it is believed that honeydew, at least that excreted by *P. citri*, contains the essential nutrients. Accordingly it is suggested that *P. citri* honeydew contains at least the following nutrients:

1. Carbohydrates.
2. Protein in a hydrolyzed state, largely free amino acids.
3. Minerals.
4. B vitamins: thiamin, nicotinic acid, folic acid and choline.
5. Male fertility factor or factors.

In another honeydew feeding test<sup>1</sup> the Mexican fruit fly, *Anastrepha ludens* (Loew) was used. The adult flies used in this experiment emerged from field collected mangoes. Fifteen flies of each sex constituted the population exposed to each diet. The flies were held in large glass jars with a cheese cloth covering the opening. Distilled water was provided in all jars by using open glass reservoirs containing an absorbent cotton pad. The ovipositional substrate was an orange colored wax shell which resembled a half section of an orange fruit. These shells were developed by McPhail and Guiza (1956).

The flies were held at a temperature of 78–80°F. The relative humidity for one test was 70–85 per cent, and for another 40–50 per cent. The flies were exposed to normal daily light fluctuations which occurred at Mexico City during April and May, 1954.

The honeydew used was excreted by *Planococcus citri* reared on potato sprouts. This honeydew was obtained at the same time as that used about three years earlier in the *D. dorsalis* experiment; thus the results obtained from this old honeydew as a diet are perhaps questionable. However, no visible growth of microorganisms could be detected in or on the honeydew but a definite darkening had occurred which may indicate that the browning reaction had taken place.

The control diet was enzymatic protein hydrolyzate of yeast plus fructose, which is an effective adult fruit fly diet (Hagen and Finney, 1950; Hagen, 1952) for inducing high egg production. A simple diet of fructose was also included for comparative purposes. The influence of the artificial and honeydew diets upon the preoviposition period, fecundity, and longevity of *A. ludens* is shown in Table II.

TABLE II. Comparative Influence of *Planococcus citri* Honeydew and Artificial Diets upon the Preoviposition Period, Fecundity, and Longevity of *Anastrepha ludens* over First 29 Days.

Diet	Minimum preoviposition period: days	Total No. eggs per 15 ♀ ♀	Mortality* ♂ ♀	
Temp. 80°F; Relative humidity 70-85 per cent				
Fructose	—	0	12	11
Honeydew**	20	349	10	3
Fructose + yeast hydrolyzate***	11	2175	10	9
Temp. 78-80°F; Relative humidity 40-50 per cent				
Sucrose	—	0	10	6
Honeydew**	23	238	2	3
Fructose + yeast hydrolyzate***	15	1780	2	3

\*A total of 15 males and 15 females were exposed to each test diet.  
\*\*The honeydew used was excreted by *Planococcus citri* on potato sprouts. This honeydew was nearly three years old, and may have changed in composition since it had become somewhat darkened. No growth of microorganisms was visible in or on the honeydew.  
\*\*\*Enzymatic protein hydrolyzate of yeast is a product of Marvin R. Thompson, Inc.

The data in Table II indicate that a diet containing four grams of enzymatic protein hydrolyzate of yeast plus six grams of fructose in ten milliliters of water was an effective diet for inducing egg production, with a total of 2175 eggs being deposited over a 29 day period, including an eleven day preoviposition period. During the same period no eggs

<sup>1</sup> This experiment was one of a series conducted in the U.S.D.A. Mexico City Fruit Fly Laboratory. I wish to express my thanks for the cooperation received from the staff and particularly to W. E. Stone and A. C. Baker for making the study possible.

were deposited by the flies exposed to the six grams of fructose plus ten ml. of water diet. The honeydew was distinctly inferior to the yeast hydrolyzate diet in the level of fecundity stimulated, and the preoviposition period was nearly twice as long; thus all the eggs were deposited during the six days between the preoviposition period and the termination of the test. Could the experiment have been extended, undoubtedly the egg deposition would have been greater. Also, if the honeydew had been fresh, the fecundity perhaps would have been more comparable to that obtained from the yeast hydrolyzate in that a shorter preoviposition period may have been obtained.

Also in Table II the data seem to indicate that the preoviposition period was slightly longer, the fecundity was lower, and the mortality was less at a lower relative humidity as compared to *A. ludens* fed similar diets at a higher relative humidity. Since honeydew is hygroscopic and fructose in the hydrolyzate diets is hygroscopic, perhaps their effectiveness was influenced by the higher relative humidity.

Supporting the view that honeydew is a nutrient source having a marked influence on fecundity is the work of Hagen (1950) on *Chrysopa plorabunda californica* Coquillett. When the green lacewing adults were fed either honeydew excreted by *P. citri* or any enzymatic protein hydrolyzate of yeast, and honey, egg production was nearly equal, while if fed only honey the fecundity was significantly lower.

Zoebelein (1956) experimenting with various honeydews particularly that of the coccid *Physokermes piceae* (Schr.) found that the longevity of some parasitic wasps was equal to or better than those fed with carbohydrates. He also reviewed some of the literature concerning the different insects that have been reported to feed upon honeydews.

The suspected complexity of honeydew composition suggested by the rough bio-assay using *D. dorsalis* adults is supported in part by recent analyses of various honeydews by paper partition chromatography. Gray (1952) found among other substances 16 free amino acids and several carbohydrates including fructose, glucose, and sucrose in the honeydew of the pineapple mealybug, *Pseudococcus brevipes* (Ckll.). He also found five amino acids in the honeydew which were not found in the food source of the mealybug. Free amino acids were found in the honeydew excreted by the crescent-marked lily aphid, *Myzus circumflexus* (Buck.), by Maltais and Auclair (1952). They found 22 free amino acids and amides including all the essential amino acids known to be required by animals for growth. The quantity of amino acids they determined as 13.2 per cent as against 35.7 per cent invert sugar.

Mittler (1953) found in the honeydew of *Tuberolachnus salignus* (Gmelin) on willow ten or eleven amino acids. Only four or five of the essential amino acids known to be required for growth and reproduction in some insects were present. Ewart and Metcalf (1956) chromatographically identified in the honeydews of *Icerya purchasi* Mask., *Coccus hesperidum* L., *C. pseudomagnoliarum* (Kuw.), *Planococcus citri* and *Saissetia oleae* (Bern.) ten free amino acids apparently qualitatively identical to all five species. Only three of the essential amino acids were present however. All five coccids were reared on citrus. They also found the sugar components in the honeydews were similar between the five species except for that of *I. purchasi*.

Apparently honeydew can vary in its composition depending upon what plant host the homopterous insect is feeding and the length of time the insect feeds. Gray (1952) found that an apparent increase of amino acids occurred in the honeydew with the increased feeding period of *P. brevipes*. Also there were usually more amino acids present in the honeydew of mealybugs feeding upon pineapple leaves than in the excreta of those feeding on pineapple fruit.

The carbohydrates found in *P. citri* honeydew excreted when the mealybugs were reared on potato sprouts were fructose, glucose, sucrose, fructomaltose, and glucose-1-phosphate (Gray and Fraenkel, 1953, 1954). Similar carbohydrate composition was found recently by Wolf and Ewart (1955) in the honeydews of two scale insects but the trisaccharides they found were melizetose in the honeydew of *Icerya purchasi* and glucosucrose in *Coccus hesperidum* honeydew.

Apparently no particular attempt has been made to analyse honeydews for vitamins, salts or minerals. However, Gray (1952) did find salts of citric acid. Hackman and Trikojus (1952) found in the honeydews of several species of the scale genus *Ceroplastes* the sugar alcohol ribitol which is a constituent of riboflavin.

The fact that honeydew can provide the necessary nutrients for egg production, at least in the tephritids tested, suggests that a supply of honeydew is perhaps essential in the fruit fly ecosystem.

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# Lotta artificiale contro le larve e le pupe della Mosca delle frutta (*Ceratitis capitata*, Wied.) nel terreno, mediante prodotti organici di sintesi, e particolarmente a base di Ettacoloro

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La Mosca delle frutta (*Ceratitis capitata*, Wied.) impropriamente denominata Mosca del Mediterraneo (Mediterranean Fruit fly), costituiva, fino a qualche anno addietro, il fattore-limite per l'incremento della frutticoltura di taluni Paesi. Ciò poteva sembrare per lo meno una esagerazione, ma sta di fatto che questo Dittero Tripaneide, con le sue malefatte, con i suoi violenti attacchi, specialmente nei Paesi a clima tropicale e sub-tropicale, dove può compiere fino a 10 e più generazioni per anno, impediva, o limitava, la coltivazione di numerose tra le piante da frutto ospiti delle larve, massimamente di quelle i cui frutti a polpa zuccherina richiamano di più la Mosca, e di talune specie e varietà di agrumi.

## LOTTA ARTIFICIALE

Per la lotta artificiale contro la *Ceratitis capitata* sono stati suggeriti o consigliati vari rimedi, che distingueremo in: a) *biologici* o *agrari* (coltivazione di varietà di fruttiferi a maturazione precoce che sfuggano all'attacco dell'insetto); b) *meccanici preventivi* (protezione dei frutti mediante l'avvolgimento in sacchetti di carta oleata o, meglio, di materia plastica (per es. polietilene), applicabili solamente nel caso in cui si vogliano difendere frutti di particolare valore e non si voglia tener conto della spesa); c) *meccanici repressivi* (raccolta e distruzione delle frutta infestate, cadute al suolo o ancora sugli alberi, eseguita scrupolosamente ogni giorno; lavorazione profonda del terreno attorno alle piante, onde interrare le pupe ed impedire che gli adulti sfarfallati possano raggiungere la superficie).

Con la scoperta degli insetticidi organici di sintesi la lotta contro la *Ceratitis capitata* è entrata in una nuova fase che si può dire risolutiva, per gli ottimi risultati raggiunti mediante l'uso di taluni prodotti.

Sorvolando sugli esperimenti compiuti dagli studiosi dal 1945 al 1955, per combattere la Mosca allo stato adulto, Bodenheimer (1945) in Palestina, Frézal (1946 e 1948) in Algeria, Gómez Clemente (1948) in Spagna, Martin (1949 e 1952) in Algeria, Di Martino (1950 e 1951) in Sicilia, Boselli (1951 e 1952) in Sardegna, Costantino (1951, 1952 e 1953) in Calabria, Avidoz e Swirski (1951 e 1952) in Israele, Rivnay (1954) in Israele, Roberti (1948) in Campania, Zocchi (1953) in Toscana, Delanoue (1953) in Tunisia, Planes Garcia (1953) in Spagna, Vieira (1951) nell'Isola di Madera (Portogallo), Leonardi (1953) in Campania, Baresi e Santocchia (1954) in Liguria, Melis (1934) in Toscana, trattiamo della lotta contro le larve mature e contro le pupe nel terreno e dei risultati da noi conseguiti in esperimenti effettuati lo scorso anno mediante l'uso di due prodotti a base di Eptacoloro: uno emulsionabile al 25% di p.a., l'altro in polvere al 6% di p.a. per trattamenti a secco.

Ravvisiamo opportuno precisare che l'idea di tentare la lotta contro le larve mature e le pupe della *Ceratitis capitata* nel terreno mediante l'uso di un prodotto organico di sintesi clorurato deriva principalmente dalla tossicità dei prodotti organo-sintetici usati per irrorazioni sulle piante contro gli adulti della Mosca, e soprattutto perchè tali prodotti oltre che uccidere insetti nocivi, distruggono anche molti insetti utili (entomoparassiti e predatori) nostri ausiliari nella lotta biologica contro molti insetti dannosi alla nostra agricoltura, determinando così la rottura dell'equilibrio biologico esistente in natura.

## LOTTA CONTRO LE LARVE MATURE E LE PUPE DELLA CERATITIS CAPITATA

Nel 1930 lo scrivente suggerì di combattere le pupe della Mosca delle frutta nel terreno mediante iniezioni di solfuro di carbonio o di solfocarbonato di potassio (Costantino 1930). Questo prodotto alla densità di 38°Bé fu consigliato anche da Del Cañizo nel 1952 diluito in acqua nella proporzione del 5%.

Il metodo però non ebbe applicazione.



Con l'avvento degli insetticidi organici di sintesi, specialmente di quelli clorurati, che somministrati sul terreno o incorporati in esso agiscono per asfissia e per contatto, con un'azione di durata più o meno lunga, il metodo ha trovato applicazione contro le larve mature, e soprattutto contro le pupe della *Ceratitis capitata*.

E' noto che le larve mature della Mosca si lasciano cadere sul terreno, dove si affondano a profondità variabili da circa 2-3 cm., fino a circa 6-8 cm., a seconda della struttura del terreno e della stagione ed ivi si trasformano in pupa rimanendo in tale stadio per un periodo di 8-30 giorni a seconda della stagione.

Rui e Bellavite (1955) contro la Mosca delle ciliege (*Rhagoletis cerasi*, L.) hanno sperimentato nel Veneto, tra l'altro, con successo, l'uso di insetticidi in polvere a base di Aldrina e di Esaclorocicloesano, sparsi sul terreno sulla superficie corrispondente alla proiezione della chioma e incorporati intimamente mediante una zappatura, allo scopo di far venire gli adulti della Mosca a contatto con gli stessi insetticidi nell'attraversare lo spessore di terreno per portarsi alla superficie.

Secondo i due Autori l'uso delle polveri insetticide da somministrarsi al terreno per la lotta contro la Mosca delle ciliege è da consigliare soprattutto là dove i ciliegeti sono siti in collina in terreni accidentati, scoscesi, inaccessibili o quasi anche ai trattori ed alle Jeeps e dove quindi non è possibile portarsi con le macchine irroratrici a motore ad alta pressione, indispensabili per potere raggiungere la chioma degli alberi; dove le frequenti precipitazioni ostacolano i trattamenti alle piante e costringono gli agricoltori a ripetere i trattamenti stessi; dove gli agricoltori non dispongono di una adeguata attrezzatura meccanica; dove, infine, manchi l'acqua occorrente per la preparazione degli insetticidi liquidi.

La somministrazione a mano delle polveri insetticide sul terreno, secondo i due Autori, ovvia a tutti gli inconvenienti esposti, garantisce, tra l'altro, un'azione polivalente e di lunga durata, oltre che contro la *Rhagoletis* anche contro altre forme ipogee, e, fatto molto importante, si evita l'alterazione dell'equilibrio biologico esistente in natura tra insetti dannosi ed entomoparassiti.

I predetti Autori hanno segnalato un inconveniente verificatosi a seguito dei trattamenti di cui sopra, cioè la morte di talune specie di uccelli insettivori che si erano cibati di insetti morti per azione degli insetticidi di contatto usati.

### ESPERIMENTI DI LOTTA CONTRO LARVE E PUPE DI *CERATITIS CAPITATA* MEDIANTE PRODOTTI A BASE DI ETTACLORO IN POLVERE SECCA ED EMULSIONE AL 25% DI P.A.

In considerazione dei risultati ottenuti da Delanoue (1949), Martelli G.M. (1949), Ryan (1950), Delmayer (1952), Delmas e Thermes (1953) negli esperimenti contro larve e pupe della *Ceratitis capitata* nel terreno mediante prodotti organici di sintesi clorurati in emulsione irrorati o in polvere per trattamenti a secco, incorporando gli insetticidi nel terreno stesso; da Günthart (1947) e Jannone (1948) contro le forme preimmaginali della *Melolontha melolontha*; da Martelli M. (1949a) contro le larve del *Pentodon punctatus*, da Ciampolini, Antonelli e Giardini (1955) contro il *Temnorrhinus mendicus*, e da Rui e Bellavite (1955) contro le pupe e gli adulti sfarfallanti dal terreno della *Rhagoletis cerasi*, abbiamo ravvisato utile compiere in Catanzaro nella estate dello scorso anno, esperimenti di lotta contro larve e pupe della *Ceratitis*, usando due prodotti a base di Ettacloro, uno in emulsione al 25% di p.a. e l'altro in polvere al 6% di p.a. per trattamenti a secco.

Giova premettere che Ettacloro o "Eptaclor" è il nome comune col quale è indicato il composto 1,4,5,6,7,8, 8-heptachloro—3<sup>a</sup>, 4, 7, 7<sup>a</sup>—tetraidro—4, 7—metanoindene.

L'Ettacloro è prodotto dalla Velsicol International Corporation, C.A. di Chicago (U.S.A.), le cui caratteristiche chimico-fisiche sono illustrate nel Bollettino n. 504-16 (1955) della stessa Società.

Il prodotto tecnico è compatibile con molti altri prodotti antiparassitari (anticrittogamici ed insetticidi) come per esempio D.D.T., ditiocarbamati, poltiglia bordolese, paratione, ottacloro o clordano, dinitrofenoli, composti cuprici, malatione, ecc., nonché con vari fertilizzanti chimici, come per esempio solfato potassico, cloruro potassico, superfosfato, solfato ammonico, nitrato ammonico, ecc.—

Questo insetticida può quindi essere mescolato con tali fertilizzanti chimici, realizzando, al tempo stesso, la duplice azione antiparassitaria e fertilizzante.

Il formulato in emulsione al 25% di p.a. si consiglia di usarlo in soluzione acquosa a dosi variabili a seconda degli insetti da combattere: generalmente di grammi 300-400 per ogni 100 litri di acqua; il formulato in polvere secca al 6% di p.a. si consiglia di usarlo per la lotta contro gli insetti terricoli nella proporzione di Kg. 30-35 di prodotto commerciale per ettaro di superficie.

Per la sperimentazione da noi effettuata furono adoperati un prodotto in emulsione al 25% di p.a. disciolto in acqua nelle proporzioni dello 0,3 e dello 0,4% (300-400 grammi di prodotto commerciale, pari a 75-100 grammi di principio attivo per ogni 100 litri di acqua), ed un prodotto in polvere secca, per impolverizzazioni sul terreno e per incorporamento nel suolo, nella proporzione di circa 3 grammi per mq. —

Le prove furono iniziate il 19 agosto 1955 in laboratorio adoperando 10 vasi di terra cotta pieni di terreno vegetale setacciato per privarlo delle parti grossolane, in ciascuno dei quali sono state collocate 10 pupe di *Ceratitis* trasformatesi da 3 giorni. Solo nel vaso n.9 furono poste 8 pupe e 10 larve mature, e nel vaso n.10, 15 larve mature di *Ceratitis*.

Le pupe furono poste sulla superficie del terreno dei singoli vasi, e poi ricoperte con uno straterello di terra dello spessore di circa 2½-3 centimetri; le larve, invece, dopo di averle collocate sulla superficie del terreno dei vasi n.9 e n. 10, si approfondarono da sole nello stesso terreno.

I singoli vasi furono ricopert mediante appositi cappucci di fitta garza bianca, trasparente, sostenuti da 4 fili incrociati di ferro zincato, e collocati nel, giardino sperimentale dell'Osservatorio.

I trattamenti furono effettuati il 27 agosto nel modo seguente:  
Sulla superficie del terreno dei vasi N. 1 e 2: impolveramento uniforme a secco con Ettacoloro al 6% di p.a.;

“ “ “ “ del vaso n.3: nulla, per controllo (testimone);  
“ “ “ “ dei vasi n.4 e 5: irrorazione uniforme mediante una soluzione acquosa allo 0,3% di Ettacoloro emulsione al 25%, adoperando una pompetta nebulizzatrice a pressione, ed erogando circa 300 c.c. per mq., ossia circa 21 c.c. per ogni vaso;  
“ “ “ “ “ “ n.6 e 7: irrorazione come sopra, ma alla concentrazione dello 0,4%;  
“ “ “ “ del vaso n.8: nulla, per controllo;  
“ “ “ “ “ “ “ 9: impolveramento come ai vasi 1 e 2;  
“ “ “ “ “ “ “ 10: “ “ come ai vasi 4 e 5.

Giornalmente furono eseguite le osservazioni per esaminare gli eventuali sfarfallamenti di adulti della *Ceratitis*, i cui risultati sono riassunti nello specchio seguente:

SPECCHIO N. 1.

Vaso n.	Adulti di <i>Ceratitis</i> sfarfallati nei singoli vasi dal 4° al 12° giorno dopo il trattamento									
	4	5	6	7	8	9	10	11	12	Totali
1	—	—	—	—	—	3 ♀ ♀ 2 ♂ ♂	—	—	—	3 ♀ ♀ — 2 ♂ ♂
2	—	—	—	—	—	3 ♀ ♀ 1 ♂	—	—	—	3 ♀ ♀ — 1 ♂
3	2 ♀ ♀ 1 ♂	—	2 ♀ ♀	—	1 ♀ 4 ♂ ♂	—	—	—	—	5 ♀ ♀ — 5 ♂ ♂
4	2 ♀ ♀ 2 ♂ ♂	—	—	—	3 ♀ ♀ 1 ♂	—	—	—	—	5 ♀ ♀ — 3 ♂ ♂
5	3 ♀ ♀ 1 ♂	—	—	—	2 ♀ ♀ 1 ♂	—	—	—	—	5 ♀ ♀ — 2 ♂ ♂
6	1 ♀ 1 ♂	—	—	—	2 ♀ ♀ 1 ♂	—	—	—	—	3 ♀ ♀ — 2 ♂ ♂
7	1 ♀	—	—	—	1 ♀ 2 ♂ ♂	—	—	—	—	2 ♀ ♀ — 2 ♂ ♂
8	3 ♀ ♀ 2 ♂ ♂	—	1 ♀ 1 ♂	—	1 ♀	1 ♀ 1 ♂	—	—	—	6 ♀ ♀ — 4 ♂ ♂
9	—	—	—	—	—	2 ♀ ♀ 1 ♂	—	—	1 ♀	3 ♀ ♀ — 1 ♂
10	—	—	—	1 ♀	—	2 ♀ ♀ 1 ♂	1 ♀ 1 ♂	—	—	4 ♀ ♀ — 2 ♂ ♂

Il giorno 11 settembre il terreno dei singoli vasi fu sottoposto ad accurata setacciatura mediante un fitto crivello a maglie di 3 mm., per potere esaminare la presenza delle pupe dalle quali non erano sfarfallati gli adulti, e di pupari, riscontrando:

Nel terreno del vaso n.	1,	n.	5	pupe morte	(50%)	e n.	5	pupari	( 50%);
" "	" "	" "	2,	" "	6	" "	" "	4	" ( 40%);
" "	" "	" "	3,	" "	—	" "	" "	10	" (100%);
" "	" "	" "	4,	" "	2	" "	" "	8	" ( 80%);
" "	" "	" "	5,	" "	3	" "	" "	7	" ( 70%);
" "	" "	" "	6,	" "	5	" "	" "	5	" ( 50%);
" "	" "	" "	7,	" "	6	" "	" "	4	" ( 40%);
" "	" "	" "	8,	" "	—	" "	" "	10	" (100%);
" "	" "	" "	9,	" "	13	" "	" "	4	" ( 22%);
" "	" "	" "	10,	" "	8	" "	" "	6	" ( 40%).

Dal prospetto si rileva, innanzi tutto, che nei vasi testimoni N.3 e 8 il terreno dei quali non è stato trattato nè con Ettiactloro in polvere nè con quello emulsionabile, da tutte le 10 pupe poste in ciascun vaso sono regolarmente sfarfallati gli adulti della *Ceratitis*. Nei vasi N.1, 2 e 9, il cui terreno è stato impolverato con l'Ettiactloro al 6% di p.a., le pupe sono morte in misura del 50, del 60 e del 72%, rispettivamente; nei vasi n.4, 5 e 10, il cui terreno è stato irrorato con Ettiactloro in emulsione al 25% di p.a., in soluzione acquosa allo 0,3%, la mortalità delle pupe è stata, rispettivamente, del 20, del 30 e del 53%; infine nei vasi n. 6 e 7, il cui terreno è stato irrorato con Ettiactloro in soluzione acquosa allo 0,4%, la mortalità delle pupe è stata, rispettivamente, del 50 e del 60%.

Questi dati dimostrano che in generale l'Ettiactloro in polvere ha esercitato sulle pupe di *Ceratitis* un'azione insetticida maggiore che non l'Ettiactloro in emulsione in soluzione allo 0,3%, e che lo stesso prodotto in soluzione acquosa allo 0,4% ha rivelato un'azione insetticida pressochè uguale a quella del formulato in polvere.

Si è, comunque, ravvisato opportuno ripetere le prove su di un maggior numero di tesi, ed il 31 agosto nel giardino sperimentale dell'Osservatorio Fitopatologico di Catanzaro, in n.27 vasi di terracotta ripieni di terreno sono state poste 20 pupe o altrettante larve mature di *Ceratitis capitata*; le pupe sono state collocate sulla superficie del terreno o affondate a circa cm. 2-3 dalla superficie; le larve mature sono state poste sulla superficie, ma esse a poco a poco si sono approfondate nel terreno e succesivamente si sono trasformate in pupe.

Dei 27 vasi adoperati, 5 sono stati lasciati quali testimoni, mentre il terreno degli altri 22, il giorno successivo (1-9-1955), è stato trattato con Ettiactloro in polvere al 6% di p.a. o con Ettiactloro in emulsione al 25% di p.a. in soluzione acquosa allo 0,3 ed allo 0,4%, come è qui appresso indicato:

- Vaso N. 1.—N. 20 pupe interrate a circa cm. 2-2½; terreno succesivamente impolverato con Ettiactloro al 6% di p.a.;
- " " 2.—N. 20 pupe interrate a circa cm. 2-2½; terreno succesivamente irrorato con Ettiactloro al 25% di p.a., in soluzione acquosa allo 0,3%;
- " " 3.—N. 20 larve mature poste sulla superficie del terreno già impolverato con Ettiactloro;
- " " 4.—N. 20 larve mature poste sulla superficie del terreno già irrorato con Ettiactloro 25% in soluzione acquosa allo 0,4%;
- " " 5.—N. 20 pupe interrate a circa cm. 3 dalla superficie in terreno nel quale era stato incorporato l'Ettiactloro in polvere sparso in precedenza;
- " " 6.—N. 20 pupe interrate a circa cm. 2-2½; terreno non trattato (*testimone*);
- " " 7.—N. 20 pupe interrate a circa cm. 3; terreno succesivamente impolverato in superficie con Ettiactloro, senza incorporarlo;
- " " 8.—N. 20 larve mature poste sulla superficie del terreno nel quale era stato già incorporato Ettiactloro in polvere;
- " " 9.—N. 20 pupe interrate a circa cm. 3; terreno succesivamente irrorato con soluzione acquosa allo 0,3% di Ettiactloro 25%;
- " " 10.—N. 20 pupe interrate a circa cm. 3; terreno non trattato (*testimone*);
- " " 11.—N. 20 pupe interrate a circa cm. 2½; terreno succesivamente impolverato in superficie con Ettiactloro, senza incorporarlo;



- Vaso N. 12.—N. 20 pupe interrate a circa cm. 3; terreno successivamente impolverato in superficie con Ettiactloro, senza incorporarlo;
- ” ” 13.—N. 20 larve mature poste sulla superficie del terreno, nel quale si sono a poco a poco approfondate. Terreno non trattato (*testimone*);
- ” ” 14.—N. 20 larve mature poste sulla superficie del terreno, che il giorno successivo è stato irrorato con soluzione acquosa allo 0,3% di Ettiactloro 25%;
- ” ” 15.—N. 20 larve mature poste sulla superficie del terreno, che non è stato poi trattato (*testimone*);
- ” ” 16.—N. 20 larve mature, poste sulla superficie del terreno sulla quale era stato sparso, senza incorporarlo, Ettiactloro in polvere al 6% di p.a.;
- ” ” 17.—N. 20 pupe, poste sulla superficie del terreno sulla quale era stato sparso, senza incorporarlo, Ettiactloro in polvere al 6% di p.a.;
- ” ” 18.—N. 20 pupe, poste sulla superficie del terreno nel quale era stato già incorporato l'Ettiactloro in polvere al 6% di p.a.;
- ” ” 19.—N. 20 pupe, poste sulla superficie del terreno che non è stato nè impolverato, nè irrorato con Ettiactloro (*testimone*);
- ” ” 20.—N. 20 pupe interrate a circa cm.  $2\frac{1}{2}$  dalla superficie del terreno, sulla quale è stata poi irrorata una soluzione acquosa allo 0,4% di Ettiactloro 25%;
- ” ” 21.—N. 20 pupe interrate a circa cm. 3; terreno successivamente impolverato con Ettiactloro al 6% di p.a.;
- ” ” 22.—N. 20 pupe poste sulla superficie del terreno e poi coperte mediante uno strato di circa 3 cm. di terreno nel quale era stato incorporato Ettiactloro in polvere al 6% di p.a.;
- ” ” 23.—N. 20 pupe poste sulla superficie del terreno e poi coperte mediante uno strato di circa cm. 3 di terreno irrorato con soluzione acquosa di Ettiactloro 25%, alla dose dello 0,4%;
- ” ” 24.—N. 20 pupe interrate a circa cm.  $2\frac{1}{2}$ —3; terreno non trattato (*testimone*);
- ” ” 25.—N. 20 pupe poste sulla superficie del terreno già impolverato superficialmente con Ettiactloro 6%;
- ” ” 26.—N. 20 pupe poste sulla superficie del terreno già irrorato, superficialmente, con soluzione acquosa allo 0,4% di Ettiactloro 25%;
- ” ” 27.—N. 20 pupe interrate a circa cm. 3; terreno successivamente impolverato, in superficie, con Ettiactloro 6%.

Le osservazioni furono iniziate il 10° giorno dopo i trattamenti, per esaminare, nei singoli vasi, ricoperti mediante appositi cilindri di garza tipo “tarlatan” bianca, sorretti da 4 fili di ferro zincato incrociati. Ogni cilindro era strettamente legato, in basso, all'orlo del vaso per impedire che gli adulti di *Ceratitis* sfarfallati dalle pupe potessero volar via.

Nello specchio seguente sono riportate le osservazioni compiute nel periodo 10–24 settembre 1955:

Dall'esame dello specchio N. 2 si rileva che:

- a.— la più alta mortalità (100%) si è verificata nel vaso n. 16, dove erano state poste le larve mature di *Ceratitis* sul terreno in precedenza impolverato con Ettiactloro al 6% di p.a., e nel vaso n. 8, dove le larve erano state poste sul terreno nel quale era stato già incorporato l'Ettiactloro in polvere;
- b.— le pupe interrate alla profondità di circa 3 cm. nel vaso N. 5, dove al terreno era stato già incorporato l'Ettiactloro in polvere, sono morte nella proporzione del 95%;
- c.— le pupe interrate alla profondità di circa cm. 2 —  $2\frac{1}{2}$  nel vaso N. 1, dove il terreno è stato successivamente impolverato in superficie con Ettiactloro, sono morte nella proporzione del 90%; la stessa percentuale di mortalità si è verificata nelle larve poste sul terreno del vaso N. 3, e nelle pupe poste sul terreno del vaso N. 18, in precedenza impolverati con Ettiactloro, nonchè nelle pupe interrate a circa 3 cm. nel terreno del vaso n. 27, successivamente impolverate, in superficie, con Ettiactloro al 6% di p.a.;
- d.— Le pupe interrate alla profondità di circa cm. 2— $2\frac{1}{2}$  nel vaso n. 2, il cui terreno è stato poi irrorato con soluzione acquosa allo 0,3% di Ettiactloro emulsione al 25% di p.a., sono morte nella proporzione dell' 85%; nella stessa proporzione sono morte le larve mature poste sul terreno del vaso n. 4, già irrorato con soluzione allo 0,4% dello stesso formulato, nonchè le pupe interrate a circa 3 cm. nel terreno del vaso n. 9, successivamente irrorato con soluzione acquosa allo 0,3% di Ettiactloro, e le pupe

SPECCHIO N. 2.

Vaso n.	tipo di trattam	Adulti di Ceratitis sfarfallati nei singoli vasi											Totali n.	morta- lità %
		10	11	12	13	14	15	16	17	18	19	20	21	22
1	polvere	—	—	—	—	—	2 ♀ ♀	—	—	—	—	—	2 ♀ ♀	90
2	liquido	—	—	—	—	2 ♀ ♀	1 ♂	—	—	—	—	—	2 ♀ ♀ 1 ♂	85
3	polvere	—	—	—	—	—	—	—	—	—	1 ♀ 1 ♂	—	1 ♀ 1 ♂	90
4	liquido	—	—	—	—	—	—	—	2 ♀ ♀ 1 ♂	—	—	—	2 ♀ ♀ 1 ♂	85
5	polvere	—	—	—	—	—	1 ♀	—	—	—	—	—	1 ♀	95
6	—	—	—	—	—	1 ♀	2 ♀ ♀	2 ♀ ♀ 3 ♂ ♂	4 ♀ ♀ 2 ♂ ♂	—	3 ♀ ♀ 2 ♂ ♂	—	12 ♀ ♀ 7 ♂ ♂	5
7	polvere	—	—	—	—	1 ♀	3 ♀ ♀ 1 ♂	—	—	—	—	—	4 ♀ ♀ 1 ♂	75
8	polvere	—	—	—	—	—	—	—	—	—	—	—	—	100
9	liquido	—	—	—	—	2 ♀ ♀ 1 ♂	—	—	—	—	—	—	2 ♀ ♀ 1 ♂	85
10	—	—	—	—	—	3 ♀ ♀ 1 ♂	4 ♀ ♀ 2 ♂ ♂	2 ♀ ♀ 2 ♂ ♂	—	—	1 ♀ 1 ♂	—	10 ♀ ♀ 8 ♂ ♂	10
11	polvere	—	—	—	—	—	2 ♀ ♀ 1 ♂	—	—	—	—	—	2 ♀ ♀ 1 ♂	85
12	polvere	—	—	—	—	1 ♀	2 ♀ ♀ ♂	1 ♀	—	—	—	—	4 ♀ ♀ 2 ♂ ♂	70
13	—	—	—	—	—	5 ♀ ♀ 3 ♂ ♂	2 ♀ ♀	2 ♀ ♀ 1 ♂	4 ♀ ♀ 3 ♂ ♂	—	—	—	13 ♀ ♀ 7 ♂ ♂	—
14	liquido	—	—	—	—	—	—	—	2 ♀ ♀ 1 ♂	2 ♀ ♀	—	—	4 ♀ ♀ 1 ♂	75

## SPECCHIO N. 2 (Séguito).

Vaso n.	tipo di trattam	Adulti di Ceratitis sfarfallati nei singoli vasi														Totali n.	morta- lità %
		10	11	12	13	14	15	16	17	18	19	20	21	22			
15	—	—	—	—	—	1 ♀	2 ♀ ♀ 1 ♂	—	6 ♀ ♀ 4 ♂ ♂	3 ♀ ♀ 1 ♂	—	—	—	12 ♀ ♀ 6 ♂ ♂	10		
16	polvere	—	—	—	—	—	—	—	—	—	—	—	—	—	100		
17	polvere	—	—	—	—	—	—	3 ♀ ♀ 2 ♂ ♂	—	—	—	—	—	3 ♀ ♀ 2 ♂ ♂	75		
18	polvere	—	—	—	—	—	—	1 ♀ 1 ♂	—	—	—	—	—	1 ♀ 1 ♂	90		
19	—	—	—	—	—	5 ♀ ♀ 3 ♂ ♂	2 ♀ ♀ 1 ♂	3 ♀ ♀ 2 ♂ ♂	2 ♀ ♀	1 ♀	—	—	—	11 ♀ ♀ 8 ♂ ♂	5		
20	liquido	—	—	—	—	1 ♀	3 ♀ ♀ 1 ♂	—	—	—	—	—	—	3 ♀ ♀ 2 ♂ ♂	75		
21	polvere	—	—	—	—	—	—	3 ♀ ♀ 2 ♂ ♂	—	—	—	—	—	3 ♀ ♀ 2 ♂ ♂	75		
22	polvere	—	—	—	—	1 ♀	2 ♀ ♀ 1 ♂	1 ♂	—	—	—	—	—	3 ♀ ♀ 2 ♂ ♂	75		
23	liquido	—	—	—	—	2 ♀ ♀	3 ♀ ♀ 2 ♂ ♂	—	1 ♀	—	—	—	—	6 ♀ ♀ 2 ♂ ♂	60		
24	—	—	—	—	—	5 ♀ ♀ 3 ♂ ♂	4 ♀ ♀ 3 ♂ ♂	2 ♀ ♀ 3 ♂ ♂	—	—	—	—	—	11 ♀ ♀ 9 ♂ ♂	—		
25	polvere	—	—	—	—	—	—	3 ♀ ♀ 2 ♂ ♂	—	—	—	—	—	3 ♀ ♀ 2 ♂ ♂	75		
26	liquido	—	—	—	—	2 ♀ ♀ 2 ♂ ♂	1 ♀ 1 ♂	1 ♀	—	—	—	—	—	4 ♀ ♀ 3 ♂ ♂	65		
27	polvere	—	—	—	—	—	—	1 ♀ 1 ♂	—	—	—	—	—	1 ♀ 1 ♂	90		

- interrate a circa cm.  $2\frac{1}{2}$  nel terreno del vaso n. 11, successivamente impolverato in superficie con l'Ettacoloro;
- e. — Le pupe interrate a circa 3 cm. nel terreno del vaso n. 7, successivamente impolverato con l'Ettacoloro, sono morte nella proporzione del 75%. Nella stessa proporzione sono morte le larve mature poste sulla superficie del terreno del vaso n. 14, successivamente irrorata con soluzione acquosa allo 0,3% di Ettacoloro; le pupe poste sulla superficie del terreno del vaso n. 17, in precedenza impolverata con Ettacoloro; le pupe interrate alla profondità di circa cm.  $2\frac{1}{2}$  nel terreno del vaso n. 20, in precedenza irrorato con soluzione acquosa allo 0,4% di Ettacoloro; le pupe interrate a circa 3 cm. nel terreno del vaso n. 21, successivamente impolverato con Ettacoloro; le pupe poste sulla superficie del terreno del vaso n. 22, e poi coperte con uno strato di circa 3 cm. di terreno al quale era stato incorporato l'Ettacoloro in polvere; le pupe poste sulla superficie del terreno del vaso n. 25, in precedenza impolverata con l'Ettacoloro;
- f. — le pupe interrate a circa 3 cm. nel terreno del vaso n. 12, successivamente impolverato, in superficie, con l'Ettacoloro, sono morte nella proporzione del 70%;
- g. — le pupe poste sulla superficie del terreno del vaso n. 26, in precedenza irrorata con soluzione allo 0,4% di Ettacoloro, sono morte nella proporzione del 65%; nella stessa proporzione sono morte le pupe poste sulla superficie del terreno del vaso n. 23, successivamente coperta con circa 3 cm. di terreno previamente irrorato con soluzione acquosa allo 0,4% di Ettacoloro;
- h. — le pupe interrate a circa 3 cm. di profondità nel terreno del vaso n. 10 (testimone), sono morte, per cause naturali, nella proporzione del 10%; nella stessa proporzione sono morte le larve mature poste sulla superficie del terreno del vaso N. 15 (testimone);
- i. — le pupe interrate a circa cm.  $2\frac{1}{2}$  nel terreno del vaso N. 6 (testimone), sono morte, per cause naturali, nella proporzione del 5%; nella stessa proporzione sono morte le pupe poste sulla superficie del terreno nel vaso N. 19 (testimoni);
- l. — dalle 20 pupe interrate a circa cm.  $2\frac{1}{2}$ -3 nel terreno del vaso n. 24 (testimone) sono sfarfallati altrettanti adulti (11 ♀ ♀ e 9 ♂ ♂) di *Ceratitis*, e le 20 larve mature poste sulla superficie del terreno nel vaso n. 13 (testimone) si sono regolarmente trasformate in pupe dando origine agli adulti (13 ♀ ♀ e 7 ♂ ♂).

Ravvisiamo utile porre in rilievo che dopo le prove il terreno dei singoli vasi è stato sottoposto a setacciatura per isolare le pupe morte ed i pupari dai quali erano sfarfallati gli adulti di *Ceratitis*, e che, particolarmente nei vasi il cui terreno era stato trattato mediante l'Ettacoloro in polvere al 6% di p.a. incorporato al terreno stesso, è stata riscontrata la presenza di adulti della Mosca morti nell'atto dello sfarfallamento, e quindi rimasti con il corpo solo parzialmente sporgente dal pupario.

Da quanto sopra ne deriva che in generale l'azione insetticida più alta è stata esercitata dall'Ettacoloro in polvere al 6% di p.a. per trattamenti a secco, sparso sulla superficie del terreno, oppure incorporato al terreno stesso, prima di porvi le pupe di *Caratitis*. L'Ettacoloro in emulsione al 25% di p.a., in soluzione acquosa allo 0,3% o allo 0,4% irrorato sul terreno prima di porvi le larve sulla superficie o le pupe interrate, oppure successivamente, ha dato risultati inferiori al formulato in polvere per trattamenti a secco.

A nostro avviso i risultati conseguiti sono abbastanza significativi, ma comunque si ravvisa opportuno ripetere le prove estendendole in pieno campo su una superficie non troppo ristretta, adottando accorgimenti che consentano di esaminare, con la maggiore possibile accuratezza, la eventuale morte delle larve cadute sul terreno trattato in precedenza o successivamente con l'Ettacoloro in polvere al 6% di p.a.; la eventuale morte di queste, lo sfarfallamento degli adulti dai pupari e la eventuale morte degli adulti stessi all'atto di venir fuori dai pupari per azione contattocida o di asfissia dell'insetticida.

Circa le possibilità di utilizzazione in pratica del metodo osiamo avanzare qualche riserva, dovuta principalmente al fatto che in non pochi frutteti o agrumeti dove è necessario combattere la *Ceratitis capitata*, sono praticate consociazioni di piante ortensi che ostacolerebbero la somministrazione al terreno degli insetticidi sia in polvere che liquidi. In questo caso riteniamo che sarebbe utile somministrare l'insetticida in polvere mescolato ai comuni fertilizzanti chimici nella stessa proporzione che ha dato a noi i migliori risultati, grazie alla lunga azione residua dell'insetticida stasso nel terreno.

Altra riserva è costituita dal timore che il prodotto organico di sintesi clorurato sperimentato possa, eventualmente, avere influenze nocive sulla carica batterica del terreno, per quanto Wilson e Choudri (1948) e Smith (1948) abbiano segnalato che la somministrazione al terreno dello isomero gamma puro dell'Esaclorocicloesano non ha alcuna influenza sulla carica batterica.

E' utile, comunque, effettuare una sperimentazione in tal senso per ovviare alle difficoltà prospettate.

Se il metodo del trattamento al terreno mediante polveri insetticide potesse affermarsi, ne risulterebbe un grande vantaggio per l'agricoltura, anche e soprattutto perchè non si verrebbe a turbare l'equilibrio biologico esistente in natura tra gli insetti nocivi alle piante e i nostri ausiliari, principalmente gli entomoparassiti.

### CONCLUSIONI

La Mosca delle frutta (*Ceratitis capitata*, Wied.) è uno dei peggiori, se non addirittura il peggiore nemico della frutticoltura dei Paesi tropicali e subtropicali dove essa è diffusa.

La lotta chimica mediante irrorazioni di insetticidi organici di sintesi sulle piante è ormai entrata in una fase risolutiva, dopo i risultati ottenuti in diversi Paesi del Mondo, soprattutto del Bacino del Mediterraneo, particolarmente interessati al gravissimo problema.

La preoccupazione relativa all'alterazione dell'equilibrio biologico esistente in natura tra insetti nocivi alle piante e loro antagonisti, soprattutto dopo l'avvento degli insetticidi organici di sintesi, ha indotto alcuni sperimentatori ad adottare il metodo dei trattamenti al terreno dei frutteti e degli agrumenti mediante impolveramento o irrorazione di prodotti insetticidi ad azione contattocida e di asfissia sulle larve, sulle pupe e sugli adulti sfarfallanti dalle pupe stesse.

Gli esperimenti da noi compiuti in tal senso in Calabria lo scorso anno in vasi di terracotta mediante l'uso di Ettiactloro in emulsione al 25% di p.a. e di Ettiactloro in polvere al 6% di p.a. per trattamenti a secco, han dato risultati soddisfacenti, soprattutto adottando questo ultimo formulato sparso sulla superficie del terreno o incorporato al terreno stesso prima che avvenga la fuoriuscita delle larve mature dai frutti inquinati e la caduta sul terreno dove avvengono le metamorfosi.

Il trattamento a secco mediante l'Ettiactloro in polvere al 6% di p.a. nella proporzione di circa 3 grammi per metro quadrato di superficie, ha dato risultati migliori della irrorazione del terreno mediante l'Ettiactloro in emulsione al 25% di p.a. in soluzione acquosa allo 0,3-0,4% nella proporzione di circa 300 centimetri cubi per metro quadrato.

Ciò è molto importante sia perchè lo spargimento degli insetticidi in polvere non richiede l'uso di attrezzi, apparecchi, ecc. potendo essere effettuato a mano, mentre il trattamento liquido richiede innanzitutto l'acqua, che non è facilmente reperibile ovunque, soprattutto nei paesi caldi, e richiede anche l'uso di pompe irroratrici o altri strumenti, e quindi un maggior impiego di mano d'opera.

Una delle difficoltà per l'adozione del metodo è rappresentata dalle consociazioni di piante ortensi esistenti in taluni frutteti ed agrumeti, che ostacolano, o impediscono addirittura, il trattamento del terreno con gli insetticidi organici di sintesi sia in polvere che liquidi. Però si prospetta la opportunità di sperimentare la miscela di polveri insetticide con i comuni fertilizzanti chimici che sogliono essere somministrati all'atto della preparazione del terreno o del trapianto degli ortaggi. E data la lunga azione residua degli insetticidi di cui si tratta, è possibile contemperare la consociazione anzidetta con la lotta contro le forme ipogee della *Ceratitis capitata*.

Ove il metodo potesse trovare larga applicazione, se ne avvantaggerebbe notevolmente la lotta naturale o biologica contro i parassiti animali delle piante, in quanto si eviterebbe il turbamento dell'equilibrio biologico esistente in natura tra nemici delle piante e loro antagonisti.

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# Possibilities of Prevention of Red Locust Plagues in Africa

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## ABSTRACT

Plagues of the Red Locust have lasted altogether almost half of the past hundred years. Continuous control of the outbreak areas, which total a very small fraction of the invasion area, should prevent any more plagues. This control is now being carried out by the International Red Locust Control Service, using modern insecticides and methods, but it is costly. Attempts are being made at ecological control, unimpeded by any vested interests in the land. There are investigations on flood, forest, farm, and fire control. The most promising for early benefits is a combination of fire-control and insecticides. More biological work is needed and is in progress. The relevance of density-dependent population theory is questionable.

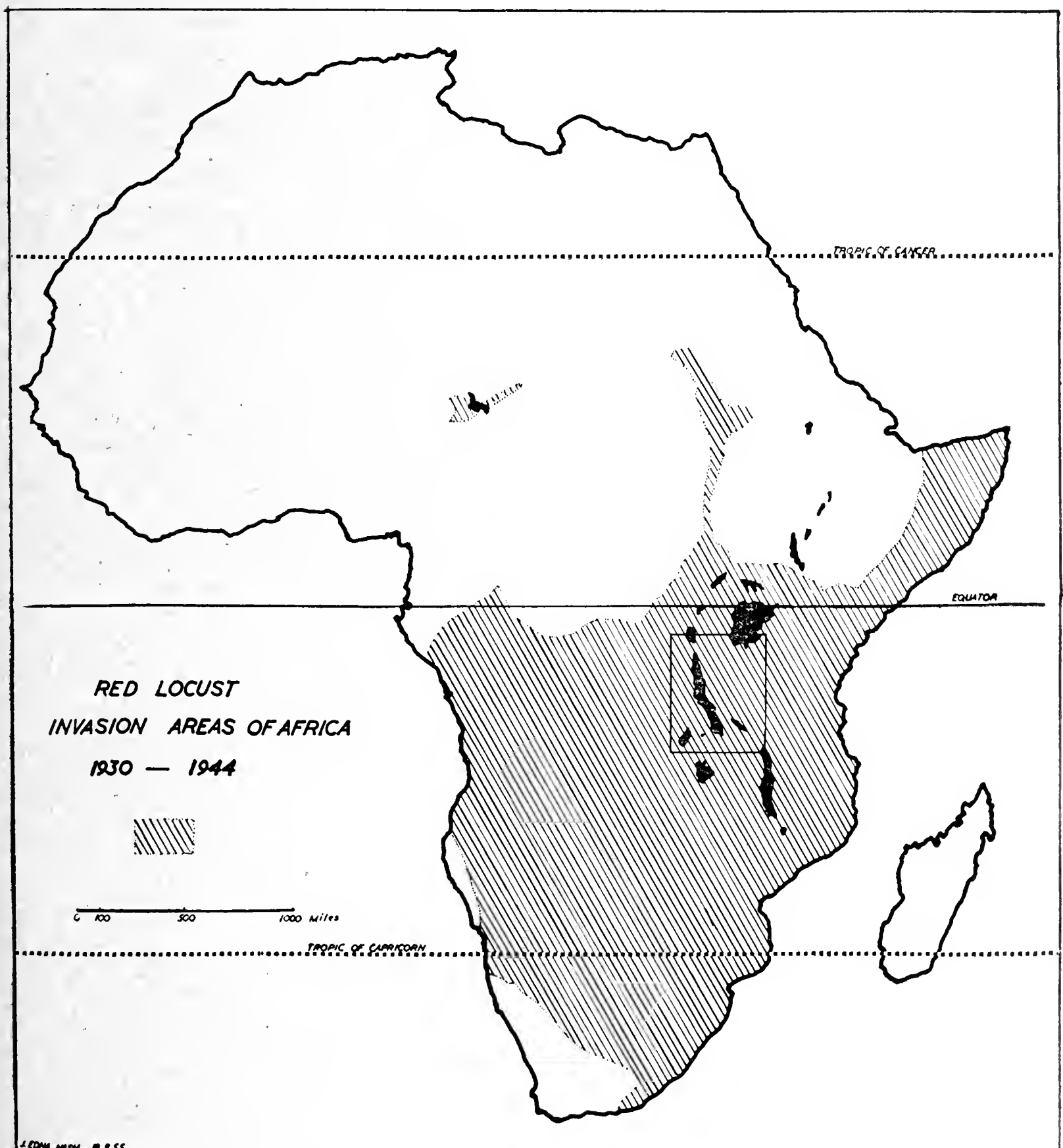


Fig. 1. The continent of Africa, showing the extent of the Red Locust plague of 1930–1944 (shaded). The rectangle includes all the recognised outbreak areas. These do not exceed 2,000 square miles in extent, compared with the invasion area of 3,000,000 square miles (after Morant 1947).

## PLAGUES

The last great plague of the Red Locust (*Nomadacris septemfasciata* Serville) began in 1929, reached a peak about 1934, and gradually died down during the following ten years (Morant, 1947). The area (Fig. 1) invaded by swarms at some time during this period was about three million square miles; that is an area as big as the United States or three quarters the size of Canada. In the middle 1930s, the Government of the Union of South Africa alone spent over a million pounds on defence of crops against Red Locusts, and quite big sums were spent in other countries too.

## THE OUTBREAK AREAS

Thanks to the initiative of Dr. Uvarov, a good deal was learnt about this plague at the time (Uvarov, 1951). It began with swarms, apparently only a few in number, which emigrated from a remote and thinly inhabited part of Northern Rhodesia into Belgian Congo; this place is called Mweru wa Ntipa, the Lake of Mud or of Marsh (Gunn, 1955). About the same time, early in 1930, at least one swarm emigrated from the Rukwa Rift Valley in Tanganyika, also going north. Like Mweru wa Ntipa, the Rukwa contains a lake surrounded by flood plains and without any outlet to the sea. The grass plains in which the locust swarms originated in these two places total about 1,500 square miles in extent. There is only one other place in Africa south of the equator in which substantial emigrant swarms are ever known to have originated from scattered populations, i.e. the Malagarasi Basin in Tanganyika (Fig. 2), and the total area involved is brought up by that to about 2,000 square miles. These, then, are the outbreak areas and their extent is less than one thousandth part of the invasion area.

The grass flood-plains which constitute the Red Locust outbreak areas have always been uninhabited and unused by men, with slight exceptions such as hunting and a few cattle in one area. To-day there is so much big game in them that they are mostly Game Reserves. Apart from that, the Control Service has virtually a free hand in them, a situation much more favourable for experiments than is enjoyed or suffered by grasshopper workers in North America. The outbreak areas are practically of no economic importance and as long as Red Locust swarms do not escape from them, nobody cares about them and nobody visits them except a few officials and ourselves.

The locust plains are generally fairly sharply bordered by forest or by lake edge. Although occasional locusts are found in glades in the forest, it is fairly certain that movement away from the plains by individuals is rare. Swarms, on the other hand, do emigrate and it is swarms that we have to stop.

## POLICY AND INTERNATIONAL AGREEMENT

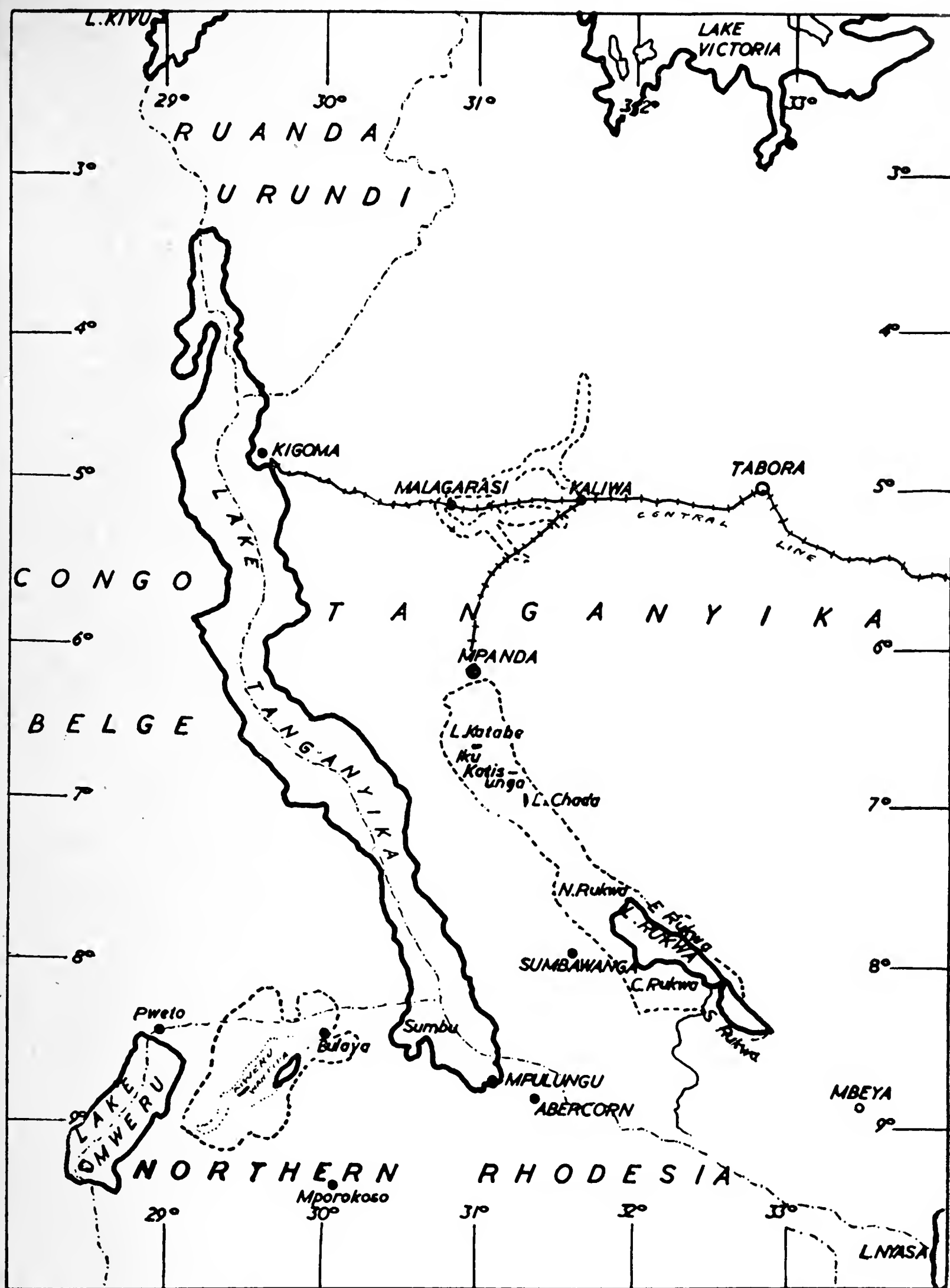
With a ratio of outbreak to invasion area of about 1,500 to 1, there is clearly a good chance that prevention of plagues in the outbreak areas would be more economical than allowing plagues to develop and then trying to protect crops. An agreement was made between the Belgians and the British in 1938 which led to the formation of the International Red Locust Control Service in 1941, which was to prevent Red Locust plagues ever starting. In 1949 a formal Convention was signed and the Contracting Governments are Belgium, Britain, Portugal, South Africa and Southern Rhodesia. This Service is now contributed to by all affected territories in continental Africa south of the equator, namely Angola, Bechuanaland, Basutoland and Swaziland, Belgian Congo and Ruanda-Urundi, Kenya, Mocambique, Northern Rhodesia, Nyasaland, South Africa, Southern Rhodesia, Tanganyika and Uganda.

## CHEMICAL CONTROL

We use chemical control against nymphs and adults. Against swarming adults, for the last couple of years we have used, almost entirely, light aircraft spraying 20% dinitro-*ortho*-cresol (D.N.C.), supplemented by a few big drift-sprayers; against hoppers we use small hand bellows dusting B.H.C., with hundreds of African casual labourers. Mr. J. Haydn Lloyd has been trying dieldrin emulsions sprayed from aircraft and they are promising.

## ECOLOGICAL CONTROL

Chemical control becomes necessary, as a last resort, when the population becomes too big. What we might try to do is to prevent the population becoming big enough for



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Fig. 2. The recognised outbreak areas of the Red Locust.

swarm formation. It is conceivable, on the other hand, that big numbers might exist without forming swarms. Anyway, if we could greatly change the ecology of the outbreak areas, perhaps no chemical control would be necessary (Gunn, 1952).

There are certain gross changes that we might make that would be likely to have the desired effect, namely flooding, afforestation, and farming.

#### FLOODING

If you can flood a locust plain and keep it flooded during the egg-laying season, that should prevent breeding. We have done one trial of that. The Iku plain is a sort of aneurism in a river flowing eventually into Lake Rukwa. The river channel disappears at one edge of the plains, spreads out over 100 square miles, and re-forms near the opposite edge. We dammed up the outflow with about 15,000 tons of soil; unfortunately, with the erratic behaviour of rain and river in East and Central Africa, the river did not flow into the plain for a couple of years after that, so we are no further forward there, except that we have a very convenient road across the dam. We are being more scientific about another trial of the same sort: conversion of the flood plains of Mweru wa Ntipa into a permanently enlarged lake by turning a biggish river into them. This scheme was originally suggested by Mr. Bredo, the first Director of the Control Service. At present a careful contour-survey is being made for the Northern Rhodesia Government, to find out how far any given amount of water would go; the river is being systematically metered, and losses by evaporation are being estimated. If successful, this flooding would convert a nuisance area into a greatly enlarged and even more profitable fishery covering about 300 square miles. Many acridids are, on the whole, favoured by dryness, except for egg laying, so even without open-water flooding, water-logging of the soil might be sufficient for our purpose.

#### AFFORESTATION

This is certainly too pretentious a word to use. It seems likely that all we need is a few trees per acre over about a million acres. Mr. Ian Robertson has been making small-scale trials to see what trees can be made to grow. The ground is salty, flooded in the rainy season, and baked hard and dry in the dry season, and the difficulty was at first to get any trees to survive at all. Some success has now been achieved with the local tamarind (*Tamarindus indica*), which survives well but does not grow, and with the exotic Persian lilac (*Melia azedarach*), which is growing well. Other trees are being tried. Noticeable extension of the natural forest has been induced by protecting it from fire in one place, but there has been little success in another. The soil profiles suggest that such natural extensions are likely to be limited by soil unsuitability. It should be noted however that the natural treelessness prevalent now may be merely a consequence of the same factors that make the environment suitable for locusts; introduced trees themselves, or the predaceous birds that they might harbour, may therefore have little effect.

#### FARMING

This, too, is too pretentious a word. The soil is mainly an intractable strongly alkaline clay, but a few species of indigenous grass are very successful. This naturally suggests cattle farming or ranching. The experts consider that the kinds of grass and their growth are suitable to carry cattle at one beast to five acres, which is a high rate of stocking for Central and East Africa. We are making a small scale trial, but there are several obstacles. Our best water pumps produce sub-soil water with one or two parts of fluoride per 100,000, at any rate at the end of the dry season. One part is marginal and two parts of fluoride are too much for continuous use by cattle. Cobalt deficiency is suspected and tests are being done. Piping water from the edges of the plains, where there is enough, would cost over £150,000 for an eventual population of 70,000 head of cattle. One could not undertake any such expense without being sure of success. Success in the cattle enterprise would depend on access to markets; the nearest railway is 150–200 miles away from the area in question (Central Rukwa) and the route passes through country infested with tse-tse fly, which carry *ngana* (sleeping sickness of cattle). It seems probable—and the cattlemen are confident—that cattle at one beast to five acres, carefully managed, would greatly change the aspect of the grass lands. Originally apparently similar lands in Kafue Flats (N.R.) and Batussi Plains (Malagarasi, T.T.) seem to have been made less favourable for locusts by grazing and trampling. But the biology of both the locusts and the cattle, and also the



economics of the scheme, need to be worked out further. It is well known, for example, that over-grazing by cattle can induce the bare spots that are so favourable to many grasshoppers; careful management would be necessary. At present the cattle are being so pestered by mosquitoes at night and tabanids in the daytime that they may not thrive as they should.

### THE GRASS MOSAIC

That brings us to more subtle possibilities, which depend on a more detailed understanding of locust biology. For a long time it has been recognized that locusts and grasshoppers are often found in a patchy habitat. Lea (1938) found Red Locusts most often in patches of tall grass within a shorter uniform stand that contained none; Key (1945) and Laurie Clark (1947) in Australia considered that a mosaic of bare patches and grass was an essential outbreak condition for *Chortoicetes*. Desmond Vesey-FitzGerald, in extensive investigation of Red Locusts in many areas, associates the adults with a mosaic of tall and short grass, and egg-laying with bare patches near tall grass. Such mosaics are very characteristic of the outbreak areas.

Vesey-FitzGerald (1955) has described, for the outbreak areas, the great zones of relatively uniform grass of one or of mixed species which are largely determined in their distribution by the incidence of flood-water: by the saltiness of water flooding back from the lake; by the comparative freshness of water spreading out from the rivers; and by the relative absence of flooding in other parts. The mosaic areas occur mostly where the zones meet; they may well be zones of instability, where a continual struggle between grass species is going on. At any rate, there you get patchiness of height of grass and there you get denser locusts.

In various species of locusts and grasshoppers, and at various times in their lives, the significance of patchiness is not always the same. The general requirements are: tall grass or trees for shelter and roosting; short grass for feeding; bare ground for basking in the sun and for egg-laying. As far as Dr. Chapman has gone in his feeding-behaviour studies on the Red Locust, it seems that the tall grass is often used for both roosting and feeding; and although the locusts spread out at times into the short grass nearby, it is not always clear why. Red Locusts never bask on bare ground; if there are a few little blades of grass, they bask on those. But they do lay eggs in bare ground. For ecological control, we are interested in the roosting in tall grass near bare ground, where the eggs are laid. How can we influence the occurrence and distribution of these bare patches?

The most interesting cause of bare patches in the Rukwa is described by Helge Backlund (1956). Some of the taller grasses, which may reach 6-8 feet in height, provide a great deal of dead leaf and stem in the dry season. This *rough*, as Backlund calls it, may accumulate until it strangles the grass which provides the rough. When the rough eventually decays or is burnt off, a bare patch remains for a short time, until it is re-colonised by the same or a different species. That gives the natural mosaic.

Although the plains are remarkably flat for hundreds of square miles, there are channels, depressions, and other irregularities in them. Consequently water is not evenly distributed over the surface. This means that grass grows better in some places than in others and, particularly, dries up sooner in some places. Then fire, the dominant factor in producing bareness, comes in.

### FIRE CONTROL

If fires begin early in the dry season, they burn patchily, partly because of pre-existing patchiness in the grass and partly because of random changes of wind. Even late fires may be patchy, if the ground has remained flooded, and you may, at egg-laying time, get clumps of tall grass surrounded by a clean burn, with the ash blown away. That is the man-made fire mosaic.

There seem, then, to be two main agencies producing good egg-laying conditions: on the one hand patchy self-strangulation of the grass, which could be prevented by burning off the rough, and on the other hand, patchy burning.

Vesey-FitzGerald's solution of this resulting dilemma depends on the fact that during the rains there is often a period of a week or so with strong sunshine and no rain. During this period, the rough can often be burnt. The growing grass is then damaged, but if the burning is done early enough in the wet season, plenty of re-growth should occur to cover the ground before the next breeding season. This burning must therefore be done as soon



as egg-laying is finished. As soon as the rough has served its purpose, it should be burnt off to prevent self-strangulation, to prevent fierce fires in the dry season, and to allow sufficient re-growth to hinder the next season's egg-laying. In fact, you don't always get a convenient sequence of events, but provided the rough does not accumulate for several years and dry-season fires can be kept out, the object should be achieved.

To make a quick and broad test of these ideas, last year a 300 square-mile self-contained area, the North Rukwa, was divided into two main parts: a fire-protected area, and a similar comparison area. Vesey-FitzGerald and Carnegie cut fire-breaks and fought fires and were completely successful in their fire control. The comparison area was burnt late in the season, in October. It did not burn clean, but left many patches of long grass amidst cleared areas. One result is clear. The tall grass that grows freely there is *sokwa* (*Echinochloa pyramidalis*) which is 6–8 feet high at best. Where large areas of this *sokwa* had been protected from fire, it contained practically no young locusts; they were found only in or near the fire-breaks, which had been bare at egg-laying time. Where the *sokwa* had been burnt late and patchily, there were locust hoppers scattered everywhere in enormous numbers. Fire protection of the mosaic zones between the main grass species does not seem to have done very much. It is within the great *sokwa* areas that it has produced such striking results. It is unfortunate that the preliminary results of Dr. Chapman's work on behaviour suggest that it is the unburnt *sokwa* that helps the locusts, as a food plus shelter habitat, to survive most readily through the long dry season.

These results might be due entirely to the locusts preferring to lay their eggs in open ground; the evidence for this preference is fairly clear, from egg-surveys. But supposing that one could abolish all bare ground in the outbreak area, would the eggs not be laid at all? If they were laid elsewhere, would they incubate and hatch less successfully? The behaviour of the locusts is not necessarily in their best interests. Those questions will be investigated but, in the meantime, we have an ecological adjunct to chemical control. By reduction of bare areas, perhaps leaving only the fire-breaks themselves bare, we can concentrate the laying and make the discovery and destruction of the hoppers much easier. This year, we shall try to prevent all fires in the tall grass.

### GENERAL BIOLOGY

The four F.'s, flood, forest, farm and fire, have been considered as possible assistants for some years. Now we are trying them. They were chosen after general and broad observations. But we must also search for hitherto unsuspected agencies. To do this, we are working towards the drawing up of a balance sheet of locust populations. The first requirement for this is to be able to count the locusts in a self-contained area.

The outbreak areas are mostly fairly self-contained, being bounded by forest edge and lake shore. You have to consider a complete area of that kind, for although there seems to be little movement out of the area, except in definite swarms, there is a great deal of individual movement within it. If, therefore, you attempt to assess what is happening to the population in part of an area, you have to take into account both movement and mortality, and when both are occurring, they are too difficult to disentangle. We are, therefore, faced with the problem of estimating the numbers of locusts in areas which range from 100 to 600 square miles in extent.

### SCOUTING

Two of us estimated locust numbers in a complete 300 square mile area three times in 1953. On each occasion, both surveyors got the same answer within a range of 10 per cent. There were then few locusts, about one in 50 square yards, and snags cropped up later, but we've made some progress. Eventually we hope to get a reasonably reliable method, so that we can find a total, subtract mortality from various causes, and see what has been happening to the locusts in a quantitative way. Methods of counting hoppers i.e. nymphs—are even more difficult, but Mr. Scheepers has made some progress there. To enable us to move in the swampy parts, we have to use a special vehicle—the swamp Skipper.

### POPULATION DYNAMICS

I think it was Sherlock Holmes who is recorded as saying that if you record all the facts, they must inevitably point to the criminal. In real life, you have to select the kinds of facts that you record. Life is too short for a compendium, as some of the synecologists

have found. How are we to decide which? Presumably one must have some general theory to guide one. Since we are dealing with a population problem, one naturally thinks of the stabilising effects of density-dependent factors. If there are important density-dependent factors which increase their pressure as a population rises, and if we could artificially reinforce them we might get an almost automatic control without insecticides.

There is little evidence that any locust has been satisfactorily controlled by predator, parasite, or disease, independently of weather. Last year we had 3,000 tons of locusts in a single area and predators had little effect. What we are quite certain of for Red Locusts, as others have shown for other species, is that weather is very important indeed, especially the exact distribution of rainfall at about egg-laying time. But Nicholson's view seems to be that there must be important density-dependent factors with every species. He has also pointed out that with swarming locusts in outbreak areas, the departure of swarms produces a marked drop in a rising population; and that, in effect, emigration is a density-dependent check on population in an outbreak area. And the Red Locust does in fact virtually die out, in the invasion area between plagues, apart from tiny struggling populations in special places. Is there any other density-dependent factor *within* an outbreak area? We have had very large populations in the last two years, but no obvious large check except chemical control, which we substitute for emigration. It is possible, of course, that an outbreak area is too big to be likened to the milk-bottle of *Drosophila* and too varied. Perhaps the locusts are always dying out in one part of the area and being recruited from a neighbouring part which has had different conditions. On the other hand, the probability theory of population dynamics of Andrewartha and Birch does seem to fit what we have observed in recent years. If we can perfect our enumeration methods, perhaps we can find out what *are* the causes of population fluctuations.

In the meantime, we have to remember that while density-dependent checks are presumed to damp down fluctuations, other kinds of check may determine the mean level of population. Although we are most interested in keeping down the peaks, if we can depress the general level enough, the peaks might not be high enough to result in emigration. We cannot control the rain, the most important factor that we know of, but we might be able to limit its effects. And a great deal more needs to be learnt of the detailed biology of the species before we can make critical trials of that kind of thing.

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# Locust Research in the Union of South Africa

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## ABSTRACT

Long-term research has been neglected during the last ten years of severe outbreaks, in favour of short-term projects on methods of control. A brief historical sketch is given of the development of control measures leading to the present methods of contact poisoning with benzene hexachloride. Brown locust outbreak dynamics are discussed in relation to rainfall. Indications are that the species increases in numbers simultaneously over vast regions, and plagues cannot be suppressed by controlling subswarming densities in relatively restricted areas.

Ecological research has been done on the relation between locust breeding and grazing practices within the outbreak region. There is no doubt that grazing practices have a profound indirect influence on the dynamics of locust outbreaks.

The main objective of behaviour studies is to determine how the degree of concentration leading to outbreaks is achieved. In ecological work the emphasis is on the influence of vegetation type, as determined by climate and man's activities, on the concentration of locusts, particularly of adults at low densities.

Research on the biology of brown locusts is directed mainly toward the study of viability and diapause in eggs, and on fecundity and survival in relation to weather conditions and food plants.

## INTRODUCTION

The outbreak region of the brown locust, *Locustana pardalina* (Wlk.), which is our most important species, covers a vast area of about 100,000 square miles in semi-arid grazing country that receives an annual rainfall of from five to fifteen inches. The carrying capacity of the land is relatively low and extensive sheep ranching is the main occupation. Farms range from about 1,000 acres in the higher rainfall eastern parts to as much as 100,000 acres in the dry north western districts of the Cape Province and South West Africa.

The prime function of the South African locust organisation is to locate and destroy all incipient outbreaks over this vast and mainly thinly populated area, to prevent migrating swarms invading the agricultural areas of the Cape, Orange Free State, and Transvaal. The present policy of the government is to shoulder the whole burden of locust control, in the interest of the country as a whole. In the early days farmers were expected to report and destroy hoppers on their land, and were assisted by the government only to the extent of being supplied free of charge with poison and pumps.

It soon became clear that the same group of farmers had regularly to carry the full burden of all locust destruction for the benefit mainly of the crop farmers beyond the boundaries of the outbreak region. Furthermore, with the best will in the world, these farmers were unable to cope with heavy and wide-spread outbreaks on their extensive farms. The government gradually took more and more of the responsibility on to its own shoulders and now, in practice, is solely responsible for all intelligence and destruction. Under the circumstances it is only natural that the main research activities of the Division of Locust Control and Research are directed towards improving intelligence and forecasting and developing more effective and economical methods of control.

A characteristic of the outbreak region is the violent fluctuation in weather conditions, especially rainfall. As a result of this the vegetation is in a dynamic state varying from sparse xerophytic shrubs of the Karoo type with very little grass during dry seasons, to a sea of grass almost smothering the bushes during exceptionally rainy summers. The locust population of the outbreak region shows a similar wide variability in numbers.

Until twenty years ago spraying with sodium arsenite by means of stirrup pumps was the standard method of control. This was superseded by baiting, using the same poison in maize bran or meal, which was much safer to both livestock and plants. The bait was scattered by hand. About ten years ago the bait was improved by the use of B.H.C. which is safer and quicker-acting. In 1949 baiting in its turn was superseded by high volume contact spraying and dusting with B.H.C. preparations.

At present, high volume spraying equipment is being used for low-volume drift spraying, and dusting with B.H.C. wettable powder is also still in use.

The scale of operations during severe locust outbreaks can be judged by the fact that in 1950/51 about £750,000 was spent. The average annual expenditure is in the neighbourhood of £200,000, but it may be as low as £30,000 in exceptionally quiet years. Poison accounts for approximately one third of the expenditure, and is the highest single item in the budget. It is obvious that great savings can be effected by the use of more economical insecticides.

### INSECTICIDE AND OPERATIONAL RESEARCH

Any promising new insecticide formulation is first compared, in the laboratory in spray or dust towers of the standard (Waters) type, with the poison in current use. The main emphasis is on formulations that would be suitable for low-volume spraying at about two gallons per acre.

A satisfactory way of determining the oil spray deposits in the cage in the bottom of the tower is to expose pairs of glass slides which are almost immediately sandwiched together for weighing. Only a very small proportion (about 10% of the spray) penetrates to the bottom of the cage in the tower after two minutes.

The most promising insecticides are then tested on a limited field scale. In order to ensure uniformity of material and environmental conditions, and adequate replication, single hopper bands are divided into a number of more or less equal portions by means of circular metal barriers each enclosing about 180 square feet. These groups of hoppers are then sprayed or dusted with three or more dosage levels of the different insecticide formulations to be tested. Mortalities are assessed from cage samples collected directly after treatment and the results studied statistically by the probit method. So far we have found no poison which can compete favourably with benzene hexachloride as regards safety in use, speed of action, and economy. Incidentally, we find that B.H.C. has an appreciable residual effect. Sprayed areas remain toxic to new arrivals for some weeks in many cases. Aldrin and dieldrin were found to be very reliable, but rather slow in action and probably not as effective as B.H.C. as contact insecticides.

Some of the organic phosphates are highly effective, but are not seriously considered because large stocks of undiluted poison would have to be handled by unskilled labourers and the toxic hazard is too great to allow their general use.

From field experiments of this nature it has been established that there is a great variation in resistance of different populations, even of the same age group, to all insecticides tested. For example, in Victoria West in 1952 it was found that required dosages were three or four times as high as was necessary to obtain comparable kills at Aberdeen six months later. It follows that there is a case to be made for an experienced officer to be kept constantly in the field during control operations, to ensure economic and effective use of the insecticide.

Great differences have been demonstrated in the relative effectiveness of B.H.C. sprays and dusts. Compared with miscible oil spray as unity, the relative dosages required to ensure equivalent kills are: wettable powder spray, 1.9; wettable powder dust, 5.5; and non-wettable dusting powder, 8.2. Thus eight times as much gamma B.H.C. is required for dusting with a non-wettable dust as would be required with a miscible oil spray. At current prices it is found to be more economical to use miscible oil sprays than wettable powders, sprays or dusts. It is also much more economical to dust with a wettable than with a non-wettable dusting powder.

Operational research in the field has amply demonstrated the practicability of using the high volume sprayers of the orchard type of which we have a large number on hand, to apply dosages as low as one or two gallons per acre. The wind, which is a fairly constant feature in the outbreak region, is used to achieve a drift of fine spray particles downwind over a swathe of 20 to 60 yards. The dosages can be adjusted by varying the swathe width or the speed of the vehicles from which the spray pumps are operated. In high winds the spray nozzles are directed at a relatively small angle and in low winds at a big angle to the horizontal. A similar technique can be adopted for knapsack pneumatic sprayers which are found most convenient for small bands or in difficult terrain. Swathe widths of three to five yards have been found practicable and satisfactory.



The rate of delivery of the orchard type sprayers is adjusted to about  $\frac{3}{4}$  to 1 gallon per minute and the pneumatic sprayers are set to deliver  $\frac{3}{4}$  to 1 pint per minute. The formula in use for determining dosage is:—

$$\text{Dosage, in pounds or gallons per acre,} = \frac{165 R}{S \times W}$$

where R = rate of emission in pounds or gallons per minute,

S = speed in miles per hour, and

W = width of swathe in yards.

## OUTBREAK DYNAMICS AND FORECASTING

Historical records indicate very strongly that brown locusts exhibit a recognisable if somewhat irregular periodicity of activity and this has been the subject of research from time to time with a view to a better understanding of the underlying reasons for this phenomenon and in the hope of arriving at some method of forecasting. The assumption was made that the most important variable in the locusts' environment is the rainfall, which is particularly variable in the outbreak region. In a recent study embracing twelve magisterial districts representative of the outbreak area, expenditure on control operations was taken as a rough index of locust numbers. Locusts were assumed to have increased significantly in numbers when the district expenditure on control in any particular season exceeded that of the previous season by a factor of three or more. Conversely, the locusts were considered as having decreased relative to the previous season if the district expenditure was a third or less than that of the previous season.

Rainfall was considered in two sections, that falling between July and December inclusive, representing the early summer rainfall, and that between January and June, inclusive, representing the late summer rainfall. On the average, March is the wettest month in the outbreak region, and the winter, from about April to August, is normally dry.

The main findings from this study of locust incidence and rainfall over twelve representative districts with four or five rainfall stations each, for the period of the last fourteen years, can be summarised as follows:

- (a) There is no close correlation between locust fluctuations and total annual rainfall or late summer rainfall.
- (b) Locust numbers are, however, very significantly related to the rainfall falling in the early part of the summer.
- (c) With few exceptions locust increase seasons are preceded by a season in which the early summer was drier than average. Years of locust decrease, on the other hand, are very regularly preceded by a season in which the early summer was wetter than average.
- (d) The old belief that locusts follow droughts is here strikingly substantiated, if by drought is meant the comparative failure of the early summer rains.

Since it is known that the early summer rains are critical for substantial grass growth, the suspicion is that locust increase is favoured by poor grass growth and retarded by good grass growth. Earlier research had indicated that locusts select short grass patches for oviposition, and that in dry seasons or in overgrazed areas the favoured oviposition sites expand, hoppers emerge in more scattered formation and swarming is delayed until the population reaches a generally high level. The resulting outbreak of killable swarms is consequently on a large scale.

## ECOLOGICAL RESEARCH

Ecological research has so far mainly been directed towards determining the effect of grazing practices on the incidence and distribution of locusts.

In an experiment laid out specially for this purpose in an area not particularly subject to locust outbreaks, it has been found that stocking at the rate of about one sheep to two acres makes the veld favourable for oviposition. More locusts are regularly found in this heavily grazed camp than in neighbouring camps which are either ungrazed or stocked at lower levels. The experiment is now in its second phase in which the previously heavily grazed camp is now ungrazed and the previously ungrazed camp is now heavily grazed. The

indications are that the locusts are behaving as expected as the vegetation is gradually responding to the altered grazing rates.

In the past sheep and goats were normally kraaled, or corralled, at night as a protection against vermin; camps were very large and water points sparse. The result was that small areas round the kraals and water points were heavily grazed, and large areas further away from water or shelter were left practically ungrazed. Under these conditions locusts no doubt increased and swarmed on relatively small patches of country. The modern practice is to stock heavily, provide relatively small jackal-proof camps each with their own water supply, and to give the stock supplementary feed during droughts. The result is that large continuous areas are now favourable to locust breeding. Not only is the area involved now much more extensive than in the past, but we also have to contend with larger total locust populations.

Other ecological research is directed towards locating relatively small ecological niches, if these exist, where the primary multiplication takes place and from where the infestation spreads into other neighbouring areas before swarming densities are attained. If such limited areas can be found it may be possible to initiate control at an earlier stage or to render such small favourable areas totally unfavourable for locust increase. In the present state of our knowledge it would appear that primary breeding areas occur on a very extensive scale. If this is true the only practical policy under the circumstances is the one generally adopted now, whereby locusts are left unhindered until they concentrate into definite dense moving bands or swarms, occupying relatively small areas, compared with the total area from which they originate. However, an immediate problem is how to satisfy land-owners in the outbreak region who are becoming more and more aware of the damage done to grazing by heavy non-swarming populations of locusts.

It is therefore all the more important to establish whether there is any possibility of preventing large population increases by applying control at a stage in the development of an outbreak much earlier than is at present considered feasible. The areas involved in primary increase may not be as large as we at present think.

### BEHAVIOUR

The behaviour of locusts is studied with particular reference to factors most likely to contribute to concentration and swarming. Behaviour connected with oviposition is of special importance.

From work already done it would seem that typical scattered solitary phase populations are relatively static. However, there is a certain amount of spontaneous movement associated with the selection of food-shelter habitats and favourable oviposition sites. This movement is rarely actually seen, probably because egg-laying is not synchronised, although the ecological requirements for oviposition may be relatively localised in space. Hatching, on the other hand, is usually synchronised by single falls of rain with the result that hoppers may be concentrated both in time and in space, and an incipient swarm may appear in an area where the parent adults had never been concentrated at any particular time.

A further factor that may contribute to numbers of hoppers appearing after rain in areas where flier populations were relatively low is the fact that brown locust eggs of solitary phase parents enter a diapause which is not necessarily broken by the first rain, thus contributing to a possible accumulation of the eggs of several generations of locusts. An accumulation of eggs in this way would be possible if successive generations were to lay a progressively smaller proportion of diapause eggs. This would happen if the succession of generations become progressively more gregarised and hence less liable to lay diapause eggs.

When adults are present at a rate of about one per one or a few square yards there is appreciable spontaneous movement which may result in population shifts of a few miles in a few weeks, but as far as we know such early *transiens* phase populations do not move very far prior to egg-laying. At this kind of density an appreciable degree of concentration takes place for mating and egg-laying and dense swarms may eventually be formed on well defined oviposition sites. Marking experiments indicated that a large egg-laying swarm of mixed age groups of phase *transiens* disperses after egg-laying and only concentrates again prior to the laying of subsequent egg-pods. The rate of dispersal and the resultant distance migrated over two to three weeks is not very great—probably about 50 miles, and not more

than 80 miles—during the late summer and early winter. Much faster movement occurs when young adult densities are of a higher order such as to permit concentration soon after reaching the adult stage and before egg-laying. Large swarms of young adults, even though they contain a high proportion of *transiens* and even *solitaria*-type green individuals, can and do migrate in dense swarms over distances of hundreds of miles in a few days before they are ready to oviposit.

The general level of activity appears to be a function of population density. Morphologically solitary phase adults are known to have migrated as members of the fastest flying swarms seen in the last twenty-five years.

## BIOLOGY AND PHYSIOLOGY

Research on the biology and physiology of locusts has been mainly directed towards elucidating trends in population levels observed in the field by means of observations on caged individuals collected from the field. This is done because it is not practicable to conduct egg surveys in the field. Advanced hoppers are collected from successive generations, fed on indigenous grasses harvested from the field or kept in cages planted over growing indigenous Karoo grasses. Notes are kept of the rate of mortality, the number of eggs laid, and the proportion of eggs in diapause. It is hoped that any great variation from generation to generation in natural mortality, fecundity, and the proportion of eggs going into diapause, will be reflected to some extent at least in the cages, which are exposed, as far as possible, to natural conditions in the Karoo. The effect of natural enemies is of course largely excluded; but the effect of climate and the state of the vegetation is not much obscured. This work will be supplemented in future by regular dissection of females caught in the field, and regular population counts over the localities from which the caged material is collected.

The effect of different indigenous grasses on longevity and fecundity is also being studied. There are indications that certain veld grasses, as compared with others, result in an acceleration of generations as well as an increased egg production. The role that food plays on the production of diapause eggs is not clear, but there are indications that eggs produced by females fed on some grasses are more difficult to hatch than those of locusts fed on other species of grass. Other cage work has indicated the importance of food fed to hoppers in determining the size and weight of the resulting adults.

In other experiments in which ovipositing locusts were given the choice between different types of soil of varying degrees of compactness, it was found that locusts can be highly selective. It was noticed that, although holes may be drilled to the full depth in almost any type of soil, the ovipositor is often withdrawn without eggs being deposited. It was clear from further experiments that egg laying is determined not by the nature of the surface soil, but by the quality of the soil at the depth at which eggs are naturally laid, that is at two or three inches below the surface.

The effect of humidity on the rate of mortality in cages has demonstrated that humidity conditions that are suitable for young hoppers are not necessarily the optimum for advanced hoppers and adults. The more advanced stages seem to require gradually drier conditions. There were also indications that under extreme humidity conditions the locusts tend to restore their own water balance by feeding selectively on dry food under humid conditions and on green food under dry conditions.

A certain amount of research is done at universities independently of the Division, and the Division encourages this by supplying locust material.

There are many other aspects of research which are relatively neglected, not because they are not important or interesting; but because the small scientific staff available is inevitably required to direct its energies to immediate practical problems connected with the control of plagues.

## DISCUSSION

R. C. RAINEY. Striking differences between kills obtained by the same control method on different occasions, as recorded by Mr. du Plessis against *Locustana*, have also been found against *Schistocerca*, in assessments both of baiting operations against the nymphs and of average spraying against the adults (see Dr. Rainey's paper in this volume. Ed.) Further systematic investigation of the factors involved in such variations in kill might well be rewarding.



# Trends in Grasshopper Research in the United States

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## ABSTRACT

*Grasshoppers in the United States are listed among the first 10 important insect pests of agriculture. Annual average damage to crops and range in the areas west of the Mississippi River has been estimated at \$75,000,000. There are some 600 species represented, of which approximately 60 are important on crops or range or both.*

*Early research gave high priority to insecticidal control with poisoned baits. The emphasis was on crop grasshoppers and the protection of field crops. With the development of the chlorinated hydrocarbon insecticides grasshopper control in crops by spraying or dusting became more or less general. Control of range grasshoppers also became economically feasible with the adoption of the new methods. Testing of insecticides has since been greatly de-emphasized and more attention is now being given to problems of biology and ecology.*

*Much of the work on insecticidal control has been concentrated in the U.S.D.A. However, the State of Montana has had a project on the ecology of grasshoppers since about 1937. This phase of grasshopper research received added emphasis in 1949 when funds from the state Entomologists' Office were made available. In 1955 a regional project was set up involving five states for work on biology, ecology, and control of range grasshoppers.*

*Present day research has undergone considerable change within the past 10 years with the added emphasis on range grasshoppers. The problem as defined in the first U.S.D.A. project outline of 1935 remains the same. The methods of attacking the problem are different. These methods are discussed.*

Grasshoppers have been rated among the ten most important insect pests in the United States. This rating is based on estimates of yearly damage to crops and range, and on the frequency with which damaging infestations occur. The general area involved comprises the States lying west of the Mississippi River, and Illinois, Michigan, and Wisconsin, east of the Mississippi. This is about two-thirds of the total land area of the United States, and includes three-fourths of the tilled land. Approximately three-fourths of the agricultural income is produced on this land.

There are some 600 species of grasshoppers in the United States. Fortunately only about 60 of this number are of economic importance. Five species attack cultivated crops, and the others are serious pests of rangelands. However, most of the crop grasshoppers are occasionally found on range and several of the range species will invade crops.

Damage by grasshoppers to cultivated crops varies greatly. In years of severe outbreaks it has approached \$100 million. According to Parker (1954), during the outbreak period 1934-39 yearly losses averaged \$63 million. From 1925 to 1949, a period which included both outbreak and non-outbreak years, the average yearly loss was about \$31 million. According to the U.S. Grasshopper Control Project, damage in 1955 was estimated at \$25 million.

In the 17 States lying west of an imaginary line from Canada to the Gulf, along the eastern borders of the Dakotas, Kansas, Nebraska, Oklahoma, and Texas, are the native grass rangelands. There are 845 million acres of grazing land in this area, which annually produce some 73 million tons of forage, which are utilized by approximately 39 million animal units (1 mature cow or 5 sheep). The value, based on the average price of beef and mutton produced from 1937 to 1950, is \$695 million annually.

Damage to range varies geographically and from year to year. It is governed largely by the grasshopper species complex, the vegetation complex, the number of grasshoppers, and the weather. Three types of damage are possible: (1) removal of forage in direct competition with livestock, (2) permanent damage to the plants due to continued grazing by grasshoppers beyond accepted percent use factors, and (3) destruction of seed heads, which prevents natural reseeding. All three types are important, but to attempt a dollar evaluation of any of them in the light of present knowledge is extremely difficult. It is our opinion, based mainly on Parker's (1930) laboratory feeding tests and our knowledge of average



population levels, that grasshoppers annually consume from 6 to 12 percent of the available forage. This amounts to between 4,673,000 and 9,983,000 tons, which would support from 2,492,000 to 5,324,000 additional animal units. The dollar value would range between \$44,376,000 and \$94,798,000.

Grasshopper research in the United States has followed rather closely the general trends of research in applied entomology, the foundation for which was developed by Riley, Walsh, Harris, Lintern, LeBaron, and Thomas about the middle of the last century (Smith 1950). Smith pointed out that "These trends are not styles but are changes brought about by new discoveries either of control materials, control methods, or of biological facts basically concerned in the work of our profession".

Abundance and outbreaks have also had an influence on grasshopper research. Parker (1940) stated that "Previous to the destructive outbreak [Rocky mountain locust, *Melanoplus spretus* (Walsh)] which was at its peak in 1876, grasshoppers seem to have been generally accepted as punishment for sinful living and nothing much was done except to cuss their activities and pray for help." In 1877 (Riley 1877) a group of governors of States and territories in which grasshoppers were destroying crops asked Congress to supply assistance in controlling the outbreak. Congress responded by appropriating \$18,000 to establish the U.S. Entomological Commission and pay the expenses of three entomologists to investigate the problem. The Commission was headed by C. V. Riley, with A. S. Packard and Cyrus Thomas as the other members. Since *Melanoplus spretus* was causing the most concern at that time, it is assumed that most of their efforts were against this species. Riley and others had used the biological approach in applied research on other insects. In the newly formed Entomological Commission Riley was assigned biology or natural history of grasshoppers and a study of their predators and parasites, Packard was assigned to their anatomy and embryology, and Thomas was given the task of determining their geographical distribution. Riley was also assigned the problem of practical control including "remedies and devices for the destruction of the locust", and Thomas that of cultural practices.

A vast amount of information was obtained by the Commission, and reports were published in 1877 and 1878. The principal methods of control developed consisted of plowing to destroy the eggs, and the use of hopper dozers and other catching machines against the nymphs and adults. The benefits that farmers derived from these early attempts at control were questionable, but a trend was established that placed artificial control efforts in high priority.

This trend was given further emphasis by the first successful use of poisoned-bran bait by Coquillett in 1885 (Riley 1891). Although Coquillett's contribution was an important step toward eventual protection of crops from grasshopper damage, it apparently did not capture the public fancy immediately. As late as 1899 L. O. Howard, then Chief of the Federal Bureau of Entomology, in a letter to a plantation owner in Mississippi, did not recommend poisoned-bran baits. This delay can probably be attributed to the low grasshopper populations that prevailed from 1879 to 1890. "Criddle mixture" was used for the first time in 1901 (Gibson 1914), and immediately was recommended generally in many States as well as in Canada. The Kansas formula (Dean 1914) made its debut in 1911 and formed the basis for the first large-scale control campaign in Kansas in 1912. From that time until chlorinated hydrocarbon insecticides came into the picture about 1945, research in practical control was centered around poisoned baits. Dozens of formulas containing various attractants, carriers, and toxic ingredients were mixed and tested, but none was more successful than straight bran containing a suitable toxicant, which is basically what Coquillett had in 1885.

During the outbreaks of 1892 and 1902 great interest was shown in the use of fungus and bacterial diseases to control grasshoppers (Parker 1940), but after extensive tests by both State and Federal entomologists this approach was pronounced a failure.

The general interest in ecology that became manifest in the early 20's had a pronounced effect on grasshopper research. Many of the State experiment stations that had conducted research on control methods turned their attention to ecological problems. The research laboratory on grasshopper control established by the U.S. Department of Agriculture at Billings, Montana, in 1922 continued to test baits for several years, but work on ecological phases was added as personnel increased. Space does not allow a complete review of the fine work accomplished between 1920 and 1945. However, the emphasis remained on grass-

hoppers in cultivated crops, and though practical control with poisoned baits was de-emphasized somewhat it still ranked high in priority.

With the coming of the new organic insecticides just prior to 1945 the trend was reversed and research in chemical control for grasshoppers was re-emphasized by both the Federal and State Governments. Much of the work was still on crop grasshoppers, but later investigations proved that several of the new insecticides applied as sprays were economical to use on the range. Grasshoppers have always been a problem with the western stockmen, but until it was clearly demonstrated that chemical control was feasible and economical few entomologists gave the problem more than a passing glance.

Range-grasshopper research was first undertaken in the Bureau of Entomology and Plant Quarantine in 1936. The principal objective was to determine the possibility of preventing, or predicting and controlling outbreaks. Studies were inaugurated to determine damage to range forage; the effects of overgrazing, weather, and other factors on populations; and the species involved and their distribution. The areas included in the study comprised most of the Great Plains from Montana to Oklahoma and northern Texas.

In 1932 Wilbur (1936) of the Kansas Agricultural Experiment Station undertook a study of grasshopper injury to inflorescence of pasture grasses. This work was followed by a study of grasshopper populations in typical bluestem pastures in Kansas, which was continued until 1939. The relationship between grasshoppers and available food plants on the range was studied by Isely (1938) in Texas and Ball (1936) in Arizona during the 1930's.

In 1940 the study of range grasshoppers was undertaken by the Wyoming Agricultural Experiment Station in cooperation with the Bureau of Entomology and Plant Quarantine. Emphasis was placed on damage to forage, food habits, nutrition, population dynamics, and control.

The Montana Agricultural Experiment Station initiated research on range grasshoppers in 1949 (Anderson 1952). Thus far emphasis has been placed on ecology, including the preferred food plants and habitats of the different species. Damage to range has also been studied, particularly the prediction of damage from the various species complexes in the different vegetative types.

In 1955 a regional experiment station project on the biology, ecology, and control of rangeland grasshoppers was approved. The contributing agencies are Montana, Idaho, Wyoming, and Colorado Agricultural Experiment Stations, the State Entomologist's Office of Montana, and the Entomology Research Branch, of the Agricultural Research Service. The objectives of this project are basically the same as those in the earlier Federal work on range grasshoppers, but the methods of approaching these objectives are quite different. In recent years there has been a trend to treat the grasshoppers more as individual species than as a complex, and this trend will undoubtedly receive greater emphasis.

Under this regional project each State formulates its own research program to fit local needs. In Idaho the effects of grasshoppers on natural and artificial reseeding of burned-over sagebrush ranges are being stressed. Specific areas for study will be permanently located, but observations will not be limited to those areas. Food-plant preferences, life history, seasonal history, and geographical distribution of species, along with factors affecting abundance, will be studied as opportunities arise.

In Colorado the studies are being undertaken on a portion of the experiment station grounds that is devoted to range improvement. The effect of range grasshoppers on the improvement experiments is being evaluated along with factors affecting populations.

In Montana the specific habitat requirements of several important grasshopper species are being determined, with major emphasis on the factors affecting populations. In addition, field studies are in progress to determine the type and amount of damage by various species at different population levels in various vegetative complexes under differing growth conditions.

There will undoubtedly be some duplication in the work of the various States and the Entomology Research Branch. The grasshopper species complex, plant associations, topography, and land use will vary from State to State.

Grasshopper research in the Entomology Research Branch is at present conducted at the Bozeman, Montana, station and its substations in Tempe, Arizona, and Columbia, Missouri. Our overall objective is to study, correlate, and evaluate factors that control or

influence populations. At Bozeman one man is assigned to biological control, another to the biology and ecology of several high-altitude range species which may have a 2-year life cycle. A problem in nutrition involving the development of a synthetic diet is also being undertaken to supply basic information on nutritional needs of *Melanoplus mexicanus*. At Tempe one man is studying the effects of alfalfa and various native plants on the survival, longevity, morphology, and reproductive potential of *mexicanus*. At present this work consists in the testing of several varieties of alfalfa as food plants. Another man is studying the range-grasshopper problem, including the role of three species in range recovery tests. This work is being done in cooperation with the Bureau of Indian Affairs of the Department of Interior on the San Carlos and Fort Apache Indian Reservations. At the Columbia substation in Missouri, studies are being conducted on the biology, ecology, and control of crop grasshoppers in that and surrounding States. The control phase, which is concerned with the timing of spray applications, methods, and materials, is being given priority.

Chemical control is still one of the most important phases of the work being conducted by the Branch. New insecticides are screened in the laboratory and then tested in the field. At present the organic phosphorus and related compounds are receiving most attention. Some work is being done with systemic insecticides. Residues from chlorinated hydrocarbon insecticides on range forage are being studied.

Much has been accomplished by all agencies, but there is still much to be done. We know far too little of the food requirements of individual species and their effect on survival and reproduction potential. We do not know enough about the biotic factors which affect populations. We are in need of new and more flexible methods for measuring populations and for measuring damage to range forage. There is still much to learn about the feeding habits of many species. We have a very efficient chemical control method for grasshoppers, but we must know our problem far better than we now do before that valuable tool can be used to the best advantage.

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# Some Recent Trends in Grasshopper Research in Canada<sup>1</sup>

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## ABSTRACT

*Recommendations for the control of pest grasshoppers in Canada by direct methods are now regarded as reasonably satisfactory. It is therefore planned that greater emphasis in research will henceforth be placed on the ecology of pest species. Of the four recognized elements of the environment that affect grasshoppers, at least some research has already been under way in Canada for a decade or more on three: weather, food, and plant and animal parasites. Studies on the effects of weather on population density, based on meteorological records and on grasshopper population data gathered during surveys, will continue, but in addition, efforts will be made to devise experimental techniques for controlled weather studies. Laboratory and field research on food plants, conducted with the aim of determining the dietary needs of grasshoppers in chemical terms, and of investigating the effects of plant species as entities in their own right, will continue, with the ultimate objective of finding the effects of nutritional factors on the reproductive rate of a grasshopper population. A survey investigation of the species distribution and percentage incidence of grasshopper parasites is now drawing to a close. There is no clear indication of what the next development in this field should be. A similar situation exists in a project for the investigation of bacterial and fungal diseases of grasshoppers, which is faced with serious inherent difficulties.*

In a recent paper, the author (1954) outlined the nature of the grasshopper problem in Western Canada, reviewed developments in control methods and referred to some of the research that has been done. The purpose of this communication is to indicate some of the current developments and lines of endeavour along which it is hoped to make progress in the next few years.

Direct methods of grasshopper control in Western Canada have now developed to a point at which those who have to make recommendations pertaining to control practices can feel that the chief limiting factors in protecting crops from grasshopper damage are probably either human nature or immediate inability to finance protection. It is expected, therefore, that barring a certain amount of more or less routine investigations on control by insecticides and by tillage, it will now be possible to increase the research attention given to some aspects of grasshopper ecology. The deliberate manipulation of some environmental elements might lead to at least partial control of grasshoppers if the means of manipulation were of a character susceptible to integration into normal farming procedures.

Andrewartha and Birch (1954) described the components of the environment of animals as weather, food, other animals, and a "place in which to live". These four exterior elements in the ecology seem a convenient framework on which to discuss much work that has been in progress on the pest species of grasshoppers in Canada, and on which to indicate lines of future endeavour.

In latitudes as far north as those of the agricultural area of Western Canada, temperatures are likely to be critical factors in limiting the "success" of any given grasshopper species. The association of weather factors with grasshopper population fluctuations has long been suspected on good evidence; recently, the relationship of weather conditions during certain critical months to abundance the succeeding year has been investigated, and the results strongly indicate significant correlations, especially between temperatures and abundance (MacCarthy, 1956). There is a great deal still to be done, using data already accumulated, and it is proposed to continue the analysis for several species in several areas. Except in a very restricted sense, weather is probably the least manageable of the four factors mentioned, but knowledge of the extent to which weather is capable of over-riding other factors that are more susceptible to management is needed. More important, is the possibility that, if the degree of regression of weather on fecundity is known, the regression factor should have utility in the preparation of forecasts. It is also proposed to test hypotheses that could be constructed on results already obtained, by developing controlled experimental studies on the effects of weather factors on reproductive capacity, which in a

<sup>1</sup> Contribution No. 3598, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.



population is of course a function of percentage survival, duration of reproductive life, and rate of reproduction. Up to now, this has been done only to the extent of setting up partially controlled experimental studies, with the grasshoppers exposed more or less to the incident weather, as it occurs from one year to the next. However, it would seem possible to develop experimental techniques, perhaps for use in the field, which to a certain extent would regulate the weather element in the environment, especially in respect of temperature, while leaving other elements, particularly food, reasonably constant. Predators and parasites would be excluded and the vegetative structure in the habitat kept as nearly optimum as our knowledge permits.

Food as a factor in the environment of grasshoppers has long been under study in Western Canada and elsewhere. It is expected that this work will continue in the Canadian grasshopper project, increasingly taking the point of view of ecology, and the aim of determining the effects of various food plants, first singly and later in various combinations, on the capacity of the grasshopper species to increase. This is now being done to a limited extent in the laboratory and the greenhouse, and in the field under caged conditions. Efforts are also being made to extend the investigation, on an observational basis, to the open field, with particular reference to recently disturbed grasshopper habitats, in which plant succession is at first rapid. There are possibilities for some control over food available to an existing grasshopper population. One pertains to certain weed species in fields of cereal grains, often inhabited by one or more pest grasshopper species. The advent of chemical methods for the control of some weeds has modified the cultivated crop land habitat to a considerable extent; there is as yet no knowledge of the effect of this on grain fields as habitats for grasshoppers. Preliminary experiments are under way now to investigate the probable effect of the deletion of herbicide-susceptible weed species from the grasshopper habitat. Another possibility pertains to the control of perennial grass species in the habitats of species that breed adjacent to cultivated fields.

The various sub-elements in the environment that may be grouped under "other animals" constitute, for Western Canadian conditions, mostly parasites. These have been under quantitative and qualitative survey in Canada for a decade or more and it is expected that the results of this long and careful work will presently be made known through publication. It may prove possible to conduct experiments with grasshopper parasites in contrived habitats. The result of such experiments could indicate how to take advantage of parasites by management practices, although avenues of approach to this end are not evident now. Along with "other animals" reference should be made to the parasitic micro-organisms and fungi, which have also been under study for about 10 years, and which are about in the same position as the dipterous parasites in respect of present lack of hypotheses concerning how they might be managed in such a way as to increase their effectiveness. Some of them, even the more promising ones, have proven intractable in laboratory study, and no new opportunities for their exploitation appear at present.

When the suitability of a "place in which to live" is considered in relation to grasshoppers in Western Canada, characteristics of the structure of the vegetation; especially height and density, are usually visualized. Generally, it is assumed that open vegetation, affording an opportunity to bask in direct sunlight while roosting on the ground, is most suitable for grasshoppers because they are commonly found in greatest numbers in such situations, but this assumption has never been submitted to an experimental test. Such a test could be arranged without much difficulty, but is not contemplated at present, except to the extent that it is proposed to keep the habitat structure in other experiments as nearly "ideal" as possible.

Ultimately, it may be possible to synthesize environments experimentally, introducing various combinations of weather elements and foods, gradually establishing, under experimental conditions, more realistic experimental habitats, but the development of experimental projects having ecological objectives is not clearly visualized in all details at present. For example, there is still much to be done in the development of methods. Objectionable as they are, field cages will probably have to continue in use in this work. Much of whatever success with simple outdoor experiments that has been enjoyed up to now has been due to simple but important modifications in the construction of field cages. As another example, only partial success has been enjoyed in rearing some species, e.g. *Camnula pellucida* (Scudd.), on foods grown in the greenhouse in midwinter at Saskatoon. The available



evidence suggests that the failure is due to the quality and/or quantity of light that enters through the glass of a greenhouse during the time of the year when the maximum angle of elevation of the sun is low. Supplementary illumination available to date has not corrected this situation, and how the difficulty is to be overcome can scarcely be imagined at present, except to use certain kinds of fresh foliage imported from sub-tropical latitudes. Such foods are known and are already in use where food is not a controlled variable in the investigation. Even objectives are not perhaps very clearly defined in every case, but it is expected that these may come into focus in due course as the work progresses and as the experimenters gain a background of experience.

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### DISCUSSION

W. A. BAKER. I would be interested in Mr. Putnam's comments on the dismal picture for use of parasites, predators and diseases in grasshopper control, in view of our present knowledge of their effectiveness against other insects and my firm belief that we have barely scratched the surface in this important field of insect control.

L. G. PUTNAM. I would agree that our knowledge at this point is insufficient to justify a position of hopelessness. However, we do seem to be "stalled", at the moment, in respect of diseases, because of certain intractable features of recognized pathogenic organisms. The position in respect of parasites is less firmly obstructed, but no clear and promising opportunities for fruitful research are evident.



# Research on the Australian Locust and Grasshopper Problem

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## ABSTRACT

Six species of *Acrididae* merit individual recognition as pests of crops and pastures in Australia. Many others must play, collectively, a significant role in reducing the carrying capacity of pastures. However, only three species have been the subject of serious research: the Australian plague locust, *Chortoicetes terminifera*; the small plague grasshopper, *Austroicetes cruciata*; and the yellow-winged locust, *Gastrimargus musicus*. The first two are major pests of wheat and pastures in southern Australia.

*Chortoicetes terminifera* is a typical locust exhibiting a well developed capacity for gregarious and migratory behaviour and showing kentromorphic phase variation. Ecologically, it is adapted to a rainfall regime with no marked concentration in either the summer or winter. Thus it has neither egg nor sexual diapause and only a moderate capacity to endure drought in either the egg or active stages. The location and ecological characteristics of its outbreak areas have been established, as well as the type of weather conducive to outbreaks. It has been concluded that the outbreak areas are particularly favourable for multiplication and not only for "concentration", and that both processes are linked with the occurrence, in a patchwork distribution, of two distinct habitats used for oviposition and for food and shelter respectively. Investigations on methods of ecological control are in progress.

*Austroicetes cruciata* is a gregarious grasshopper showing phase variation. It is adapted to a winter rainfall regime by means of a summer egg diapause and has a single spring generation per year. The ecological conditions for outbreaks have been investigated.

Six species of *Acrididae* merit individual recognition as pests of crops and pastures in Australia, and many others must play, collectively, a significant role in reducing the carrying capacity of pastures. Of the six, *Locusta migratoria* L. and *Austracris guttulosa* (Walk.) are sporadic pests in northern Australia, the first damaging sugar-cane and the second mainly pastures; and *Phaulacridium vittatum* (Sjöst.) is a local pest of fruit trees, vegetables, and tobacco in southern Australia. No serious research has been carried out on these three. A fourth species, *Gastrimargus musicus* (Fabr.), a pest of pastures and gramineous crops in northern Australia, has been investigated by Common (1948). The remaining two species, *Chortoicetes terminifera* (Walk.) and *Austroicetes cruciata* (Sauss.) are major pests of wheat and pastures in southern Australia and have been studied extensively since the early 1930's by workers in the C.S.I.R.O. and the Waite Institute, University of Adelaide.

*Chortoicetes terminifera* (the "Australian plague locust") is a true locust of rather slender build and distinctive appearance. Taxonomically it is related to *Locusta* and *Locustana*, among the Old World locusts, rather than to *Schistocerca* or *Nomadacris*; however, it is decidedly smaller than any of these, though larger than the New World grasshoppers *Melanoplus mexicanus* and *Camnula pellucida*. It forms typical, strongly gregarious swarms in both hopper (Clark, 1949) and adult stages. The adult swarms may migrate for distances up to 500 miles, but more usually for only 50–100 miles. Kentromorphic<sup>1</sup> phase variation is well developed in the adults; thus absolute size, sexual dimorphism in size, and the tegmen/femur ratio all differ characteristically as between swarming and non-swarming individuals (Key, 1954).

Ecologically, *Chortoicetes* is adapted to a rainfall regime with no marked concentration in either summer or winter. Thus it has neither egg nor sexual diapause and only a moderate capacity to endure drought in either the egg or active stages. The eggs, for example, begin to show high mortality after about three months if moisture is inadequate for hatching and the temperature favourable (Key, 1942). These characteristics are responsible for the occurrence of a variable number of annual generations from one to three and a partial fourth, according to the amount and distribution of the rainfall in particular years.

The epidemiology of *Chortoicetes* has been studied in a number of papers by Key and Clark of C.S.I.R.O. (see particularly Key, 1945, and Clark, 1947a) and Andrewartha and

<sup>1</sup> A term introduced in a paper by Key & Day (1954a) to denote phase variation in Uvarov's sense.

his associates at the Waite Institute (Andrewartha, 1940). These have established the location and ecological characteristics of its outbreak areas and the type of weather conducive to outbreaks. The outbreak areas are quite numerous, more than 20 having been recognised, and rather extensive in total area. Thus they constitute a much larger fraction of the infestation area than do the outbreak areas of *Locusta* or *Nomadacris* and are perhaps more comparable with those of *Locustana*. The most active ones are situated in grazing country in New South Wales, which is also the State where the bulk of the damage is suffered. Migration takes place in a predominantly coastward direction, i.e. eastwards in northern New South Wales and southern Queensland, through south-eastwards to southwards and south-westwards in western New South Wales, Victoria, and South Australia. It is mainly of the "rolling" type, but flights at several thousand feet have been recorded. Although no adequate analysis of flight directions has been made, the determining factor is probably the direction of hot, strong winds, which is consistently coastward in south-eastern Australia.

It has been shown that the major outbreak areas are situated within a climatic zone particularly favourable, in its temperature and moisture characteristics, for locust survival and multiplication. Within this zone they are confined to cleared or naturally treeless country, usually very flat, in which there is a patchwork distribution of heavy "self-mulching" and lighter compact soils. The self-mulching soils occur at slightly lower levels and bear a characteristic vegetation of spaced large grass tussocks, while the compact soils occupy the higher levels and have, under normal grazing, a shorter and less tussocky grass cover which is apt to develop bare patches. The habitat represented by the self-mulching soil and its typical vegetation is preferred by the active stages of the locust. We have concluded that this is because of the food and shelter it affords the insects when these requirements are deficient elsewhere, and we have termed the habitat the *food-shelter habitat*. Bare patches amongst low cover on the compact soils are selected for oviposition, and we have termed this the *oviposition habitat*.

The favourableness of the outbreak areas for locust survival and for rapid multiplication under favourable weather conditions may be explained on the basis that the mosaic of soil types, and hence of the food-shelter and oviposition habitats, ensures that individual locusts will always have available, within their normal range of dispersal, conditions favourable to their stage of development, whatever that may be. The mosaic environment is strongly buffered against the effects of climatic variation. The effect of a wet period is to expand the food-shelter habitat at the expense of the oviposition habitat, but without entirely eliminating the latter; and the effect of a dry period is to expand the oviposition habitat without eliminating the food-shelter habitat. Portions of the two habitats that are particularly resistant to change have been termed food-shelter and oviposition *nuclei*.

The almost continual fluctuation in the relative areas of the food-shelter and oviposition habitats frequently leads to a marked concentration of locusts in one or other habitat which for the moment is in short supply, and may thus set in train the development of gregariousness. Wherever a food-shelter nucleus and an oviposition nucleus are situated close together, we have particularly favourable conditions both for multiplication and concentration, and it is here that swarms are formed. Such situations therefore constitute the outbreak centres of *Chortoicetes*.

Australian workers have tended to emphasise the somewhat neglected role of the outbreak areas in favouring survival and multiplication of locusts and to minimise the significance of the classical phase characters in the outbreak process (Key, 1950). They have also devoted much attention to the relation between outbreaks and weather. Without entering into the controversy on the relative significance of density-dependent and density-independent factors in determining levels of abundance, we may say that weather, and especially rainfall, is the independent variable to which fluctuations in the abundance of *Chortoicetes* must for the most part be ascribed, although, obviously, the influence of this factor is largely indirect and exercised through the responses of the food and shelter factors and of natural enemies, especially diseases. It has been possible to delineate "outbreak provinces" in each of which a distinct type of departure from the normal rainfall regime can be expected to favour multiplication in the outbreak areas of the province. Thus in one province the summer rainfall needs to be higher than normal, in another lower (Key, 1945).

The general trend towards the use of organic insecticides and the substitution of sprays for baits in locust and grasshopper control has been followed also in Australia.

Direct defence has hitherto been the only strategy employed. However, the knowledge necessary for outbreak suppression has long been available and arrangements have recently been made to test this strategy against *Chortoicetes*. For a number of years the C.S.I.R.O. has been investigating possible methods of ecological control in the outbreak areas, i.e. on the possibility of altering their ecological characteristics in such a way that swarms no longer form in them and outbreaks are prevented. Any practical recommendations that may emerge from this work are likely to involve the planting of critical areas with trees or shrubs. The work of Clark (1950) has shown that *Chortoicetes* avoids entering stands of timber, and we also know that its density is always low even in stands that are very far from casting continuous shade.

*Austroicetes cruciata* (the "small plague grasshopper"), although related taxonomically to *Chortoicetes*, is a very different insect. In many features of its biology and ecology it is reminiscent of the Moroccan locust, *Docostaurus maroccanus* Thunb.—no doubt a case of convergence under the influence of a similar, Mediterranean-type environment. It is quite a small species, although proportionately more robust than *Chortoicetes terminifera*, and lacks the characteristic black spot on the tip of the hind wing of the latter. *A. cruciata* must be classed as a gregarious grasshopper showing phase variation. It may form fairly compact swarms in the hopper stage, but the adult swarms are loose and only weakly migratory, probably rarely moving as much as 15 miles from their point of origin. Phase variation is striking in the male, hardly evident in the female (Key, 1954). Males from swarms are considerably larger and tend to have a higher tegmen/femur ratio; when sexually mature they are bright yellow, as in the gregarious phase of a number of locusts but not of *Chortoicetes*. The sexual dimorphism is also less in swarming populations. The males have the habit of clustering around females in the act of oviposition, forming little groups that are very conspicuous when the males are yellow.

The differences between *A. cruciata* and *C. terminifera* are most clearly expressed in their ecological adaptations. *A. cruciata* has a much more restricted distribution range than the locust, being confined to a semi-arid region in southern Australia with a predominantly winter rainfall. The eggs are laid in the late spring and early summer and remain in diapause throughout the summer and following winter, hatching in the very early spring after nine months in the ground (Andrewartha, 1943a). The function of this protracted diapause is clearly to prevent the hatching of eggs in response to light summer rain in the winter-rainfall region, when food would be unlikely to be adequate for development of the resulting nymphs. Thus the species has a single annual generation, which appears very early and takes advantage of the flush of feed produced when temperatures begin to rise after the winter rains.

The weak migratory capacity of *A. cruciata* confers on its outbreaks the character typical of a grasshopper. That is, the increase in numbers occurs within the areas where the damage is suffered and may or may not involve actual swarm-formation, so that there is no differentiation of outbreak areas and invasion area, although some portions of the area liable to infestation are undoubtedly more favourable for multiplication than others. The infestation area coincides with the drier section of the wheat belts of Western Australia, South Australia, and Victoria, and in New South Wales includes a considerable amount of pastoral country as well.

The characteristics of the infestation area have been studied by Andrewartha (1944) and Clark (1947b). It is limited inland by the frequency of droughts, which cause high mortality of hoppers and adults from starvation, and coastward by over-moist conditions favouring disease. Swarms and other dense populations are confined to treeless areas of compact soil with an open, mainly annual pasture. Sandy and self-mulching soils are avoided, although they may be invaded by swarms making their limited migrations from adjacent compact soils. The avoidance of timber and shrubby vegetation by this grasshopper is as marked as that of *Chortoicetes*, and wide areas that were quite unsuitable for it in their virgin condition have been made favourable, especially in Western Australia, by clearing for wheat cultivation and subsequent abandonment to pasture.

Thus the whole life-cycle of *A. cruciata* is passed in the type of habitat which in *Chortoicetes* we have recognized as the oviposition habitat. It is more intolerant than the latter species of the denser type of pasture developed on the self-mulching soils, perhaps because it occurs at a time of year when temperatures are relatively low and humidities high



and hence when facilities for basking are more important than shelter. Nevertheless, within this compact-soil habitat, sites may be distinguished that appear to serve as oviposition and food-shelter habitats for the grasshopper; but the food-shelter habitat bears a lower and more open cover than that of *Chortoicetes*.

Outbreaks of *A. cruciata* tend to follow years of intermediate rainfall in the infestation area—rainfall adequate in amount and distribution to provide green feed for hoppers and adults from hatching to oviposition, but not so high as to produce a tall, dense growth of pasture. It is in the drier of such years that damage from a given level of infestation is greatest, for then the pastures are less able to withstand the attack and crops are more likely to be invaded.

The same control methods are used against this grasshopper as against *Chortoicetes*, except that in Western Australia considerable importance is attached to the ploughing of egg-beds as a means of destroying the eggs and starving out the hoppers that hatch. The strategy of outbreak suppression is of course not applicable to a grasshopper. Andrewartha (1943b) has advocated the preventive control or mitigation of the pest by the substitution of perennial pastures of browse shrubs for the present pastures of ephemeral annuals, but this suggestion does not seem to have been taken up.

*Gastrimargus musicus* (the "yellow-winged locust") is a large species with a bright yellow hind wing with black band. It is closely related to *Locusta* and *Locustana* and about the same size as the latter. In northern Australia it forms dense swarms that migrate hundreds of miles. It may also become numerous, but without forming swarms, in south-eastern New South Wales and eastern Victoria, causing slight damage to pastures and gardens. Thus, although in the south it behaves as a grasshopper, in the north it shows itself a true locust. Phase variation is well developed in both nymphs and adults, where it involves the tegmen/femur ratio, the pronotum/head ratios, and the sexual dimorphism in tegmen length (Common, 1948). Sexually mature gregarious males are not yellow. Although *Gastrimargus* is a large Ethiopian and Asiatic genus, this appears to be the only species for which phase variation has been reported.

*G. musicus* is primarily adapted to a summer rainfall. In northern Australia it has two annual generations, at least during outbreaks, and apparently no diapause. The development of outbreaks has been studied only in Queensland, where the most frequently infested region lies inland from Mackay and Rockhampton (Common, 1948). The swarms appear to originate in an outbreak area in the general neighbourhood of Clermont, from which they spread in a coastward direction during successive seasons. The precise limits of this outbreak area are not known, nor the characteristics responsible for its capacity to produce swarms. It must be assumed that the outbreaks recorded from the Northern Territory and the Kimberley district of Western Australia originate in outbreak areas situated in those territories, but their exact location is unknown.

The only attempts at control of *Gastrimargus* have been of the direct defence type. It is evident that outbreak suppression could be attempted if further research were carried out on the location of the outbreak areas; outbreak prevention is also theoretically possible.

Apart from research directed specifically at the three pest species we have discussed, work of a more general kind is also proceeding. The taxonomy of the Australian Acridoidea is still very poorly known. Indeed, it is quite likely that less than half of the existing species have been described. A comprehensive revision is being undertaken by Rehn, of which two volumes have already been published (Rehn, 1952, 1953), and this is being supplemented by studies on particular groups of genera (cf. Key, 1954). A survey of the host-relations and distribution of ectoparasitic mites is being carried out by C.S.I.R.O. Key and Day (1954a, b) have recently published two papers on a physiological colour response in an alpine grasshopper, *Kosciuscola tristis*, which is sometimes very abundant at higher elevations in the Australian Alps.

In the field of insecticides, investigators in the various State departments of agriculture are continually examining new preparations and methods of distribution, the New South Wales Department of Agriculture concentrating particularly on various power sprayers for ground operation (Anon., 1954), and the Victorian Department of Agriculture on distribution from aircraft (Hogan, 1952).

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# Recent Trends and Needs of Acridological Research

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## ABSTRACT

*Large-scale insecticidal control of locusts and grasshoppers has proved to be both effective and economically sound for the protection of standing crops and pastures.*

*Successes of chemical control are short-term in nature, and the treatment must be repeated whenever the pest population reaches dangerous proportions. Nevertheless, its immediate results have a sufficient appeal to cause a concentration of efforts on further improvement of techniques, while research directed to a better understanding of the problem and to its long-term solution is lagging behind.*

*A long-term approach requires systematically organized investigations on each species throughout its whole distribution area, regardless of boundaries.*

*The main items in a long-term research programme on each species are: taxonomy (including intraspecific variation); geographical distribution; ecology of each stage in the life-cycle; behaviour; physiology of survival and of reproduction; population dynamics; effects of land-use practices (examples will be given illustrating recent progress in the different directions and the main gaps in knowledge).*

*The locust and grasshopper problems of the world are increasing in economic importance with the development of new regions. Chemical control of these pests should be regarded as offering only a temporary respite greatly needed for developing long-term research, without which the ultimate solution of these problems cannot be attained.*

## INTRODUCTION

There is no doubt that large-scale control of locusts and grasshoppers by modern insecticides is both effective and economically sound as a means of immediate protection of crops and pastures. Continuous progress in developing new insecticides and in perfecting the technique of their application should lead to an even greater efficiency, possibly at less cost. However, the successes of chemical control are inevitably short term ones and the treatment must be repeated whenever the pest population reaches dangerous proportions. Nevertheless, its immediate results are so obvious that farmers and governments are easily persuaded to bear the costs, even if they become recurrent. This has resulted in pressure on entomologists to concentrate their efforts on further improvements of control techniques, to the detriment of research directed to a better understanding of the problem. Research on the problem itself, not merely on the measures temporarily to reduce its consequences, finds only limited support, although its aim is a long-term solution of the problem and the elimination of a threat which otherwise becomes chronic.

It is my intention to review briefly the present state of long-term acridological research, underlining the gaps in our knowledge. It is hoped that my emphasis of the shortcomings of research in different directions will be taken not as a wish to criticise, but as an attempt to outline the complexity of the problem and to suggest some lines of approach which appear promising but require more attention than they have received in the past.

## COMPREHENSIVE APPROACH

An essential feature of a comprehensive investigation of any insect species should be its long-term study throughout the whole distribution area, not merely where and when it becomes a serious pest, since only such an approach affords a hope of discovering the reasons for its economic status. This view was at the basis of the historical attempt of the United States Entomological Commission, the creation of which "was but an expression of the public demand for more light on the locust problem, which was to a great degree involved in darkness and mystery. Investigation was called for because it was felt how little of a definite and satisfactory nature was generally known on the subject". The approach of the Commission to their work was an all-embracing one and much knowledge was acquired on the Rocky Mountain Locust, but unfortunately, this work was of a temporary nature and in the following 75 years only short-term and localised studies were

carried out in the North American continent. It is only quite recently that an inter-state project has come to life, but its scope and scale are still well below the size of the problem.

A comprehensive approach was adopted in Australia, and notable advances have been achieved towards understanding the problem and its causation. In Africa and south-western Asia, continuous studies of three locust species have been in progress for the last 25 years and in the case of two species, the results have been rewarding. In South Africa, intensive studies of the Brown Locust have made good progress, but they have had to give way lately to pressure for short-term chemical control.

Apart from the problems of individual species and regions, there is a need for comparative studies, and for organised exchange of experience. No provision for this essential need exists.

I will now turn to a review of the different aspects of a comprehensive investigation.

## TAXONOMY

It is a surprising fact that the specific identity of some of the economically important locusts and grasshoppers is still insufficiently known. There is, for example, some controversy with regard to the species of *Schistocerca* in South and Central America; the European and West Asian species of *Calliptamus* are in confusion; and the identification of range grasshoppers in the Americas has only recently made progress. The value of taxonomic work was clearly demonstrated in Australia where several closely allied species of two genera have been methodically studied and their different economic status established. In North America, it was only quite recently discovered that more species than one have been involved in plagues of swarming *Melanoplus*; this makes difficult the study of earlier, and even of recent, records.

Apart from species taxonomy, there is a need for comprehensive studies on geographical subspecies. Such work, e.g., on *Locusta migratoria* (L.), has revealed the existence of several races, differing in their swarming tendencies and therefore in economic importance. The Desert Locust, *Schistocerca gregaria* (Forsk.), notorious for its swarming abilities in the northern half of Africa and western Asia, is represented in South Africa by what is, apparently, a distinct race that swarms only rarely and is not a recognised pest—at least, so far.

These examples lead us to the problem of phase variation, that is, variation connected with population densities. There has been much more theoretical controversy on the subject, than critical study of it. Recent work indicates that the importance of some aspects of phase variation may have been overestimated in the past, but no study of population dynamics of swarming species can be complete unless phase variation in behaviour and physiology is taken into account. The effects of density on behaviour, ecology and population dynamics of normally non-swarming grasshoppers are suspected, but largely unstudied; this is no reason to assume their non-existence.

## GEOGRAPHICAL DISTRIBUTION

In most species, the area of economic importance is only a part of the general distribution area. It is obviously essential to know the latter in order to elucidate the conditions responsible for the difference, but this approach is seldom attempted. In the case of migratory locusts, the geographical patterns of migration have been consistently and objectively studied only with regard to the three African species; in South America, some seasonal migration patterns have been suggested, but they have been based on short-period data referring only to parts of the whole area. With regard to grasshoppers, Russian work on Siberian grasshoppers indicated possibilities of distinguishing the range of economic importance from the general distribution area.

## ECOLOGY

The essential feature of acridid ecology is that the main requirements of the active instars, hoppers and adults are food and shelter provided by plants, whereas their eggs are laid in soil, mainly outside dense vegetation cover. A habitat should satisfy all needs and it is an almost general rule that the economic species are connected with habitats characterised by a mosaic of plant cover and bare soil. Such habitats are normal in natural ecotones, but are also created by human activities—cultivation and, especially, uncontrolled grazing.



A considerable amount of ecological investigation on individual species has been carried out, but the majority still await detailed and critical investigation.

Possibly the main criticism that can be directed against much of the previous work on ecology of Acrididae is that too much attention has been directed to synecological studies while the ecology of component species of supposed associations has been largely ignored. Detailed autecological studies, enabling comparisons to be made between different species, are more likely to advance the knowledge of the species studied, and to provide reliable data for generalisations, than a synecological approach in which species tend to lose their identity.

### BEHAVIOUR

The ecology of Acrididae is closely dependent on their behaviour, since the complex nature of their habitat imposes on them the need for movements from one part of it to another. Indeed, movements inside the habitat are very typical of most grasshoppers, even those unable to fly (hoppers, brachypterous species), but remain almost unstudied. Movements leading to a change of habitat or those connected with its expansion and contraction due to seasonal changes in ecological conditions, are of obvious theoretical and practical importance, but very few examples of such work can be found. In migratory locusts such movements follow well established seasonal cycles, which, in the Desert Locust, have proved to be closely connected with dynamics of weather systems; comparable work on other locusts is lacking.

An important feature of flight activity of the Desert Locust and the African Migratory Locust is that, while their swarms fly largely in day-time, the non-swarmling populations do so at night. Night flights are also of common occurrence amongst many grasshoppers in climates where the nights are sufficiently warm; they may play a prominent part in population movements, but remain practically unstudied, or even ignored.

With regard to feeding activities, some very recent studies have revealed a selectivity with regard to food plants, the degree of which varies between species; as a result, e.g. some grasshopper species abundant in pastures and previously considered as pests, proved to be feeding on plants of no value as grazing for animals. Work of this kind is still inadequate but it is essential, particularly in the case of range grasshoppers.

A most important feature of locust behaviour is gregariousness. In fact, the gregarious habit is the only criterion by which locusts are distinguished from grasshoppers, and the swarming phases of locusts from the solitary. Also it is the gregariousness of locusts that makes them particularly serious pests, as it results in the assembling of millions of individuals in swarms liable to move for very long distances and carry out concentrated attacks on crops. Field studies on gregarious behaviour has been carried out only recently, mainly on the Desert Locust, and they have revealed the great complexity of the causal factors of gregariousness, both ecological and physiological. Laboratory studies on gregarious behaviour in this and other locusts have shown that the habit of aggregation and of subsequent coherent behaviour is a matter of conditioning during hopper life. The response to conditioning is a specific one; some species acquire the habit in a matter of hours, while others require several days. Some grasshopper species, normally non-swarmling, have been known to occur in marching bands and flying swarms, thus exhibiting an occasional gregariousness which affects their mobility. On the other hand, certain other grasshopper species fail to show any effect of density on their behaviour, and all gradations between the two types may be expected to be found, when attention is paid to such studies.

### PHYSIOLOGY

Behaviour studies inevitably lead to the need for physiological knowledge. The mechanism of movement, particularly of flight, has been studied in detail, including its aerodynamic and metabolic aspects, but only in the Desert Locust, and these studies have thrown much light on its migrations, which constitute an important part of its biology.

Some types of movement may be spontaneous, that is, due to internal rhythm or physiological stimuli, but they remain little investigated, except with regard to their limiting external conditions. Even more serious is the present very low state of knowledge of acridid sensory reactions. Indeed, while some experimental and observational evidence on sensory responses is available, studies on the physiology of sense organs are still in their infancy. Moreover, while many sense organs are known anatomically, the function of the

majority of them is still a matter of speculation. Studies on vision, hearing, olfaction and other senses in Acrididae are sorely needed for the interpretation of their behaviour.

Another aspect of acridid physiology in which progress is required in the interests of a better understanding of their biology and ecology is nutrition. The effects of food plants on survival and, particularly, reproduction have been the subject of some purely empirical studies, but the physiological basis of nutrition cannot be attacked until an adequate synthetic diet has been produced, making possible fundamental studies on nutritional requirements of individual species. Such work is only in its initial stages, and much greater and wider efforts are required.

The physiology of reproduction is another problem of major importance, but, apart from intensive laboratory work on African locusts, very little attention has been paid to it. The work on locusts has provided valuable data, particularly with regard to their sexual maturation, fecundity, and fertility, as affected by physical environment and, what is particularly interesting, by the population density. The latter effect, in the case of sexual maturation, was shown to be probably under hormonal control evoked by the proximity of other individuals, thus linking up sensory perception and nervous transmission with hormonal production and its metabolic effects. While of great practical importance, these results suggest that locusts may be exceptionally suitable for investigations of certain fundamental physiological problems.

Again, the physiology of development of Acrididae presents a number of problems, few of which have been sufficiently investigated. The aspect that has received much attention is the embryonic development and, particularly, the egg diapause. It is noteworthy, however, that in spite of the vast amount of work done, the metabolic aspect of diapause is still far from being clear. Also, most of this work was conducted on acridids from temperate climates, while embryonic development and egg diapause in subtropical species, subject to long dry seasons, remain unstudied.

The physiology of growth is still little known, although recent work on locusts has suggested a most intriguing connection between the rate of development, including the number of instars, and population density, i.e. still another phase effect. Even more interesting is the finding that the rate of growth, as well as the size, colouration, and morphology of progeny is affected by the density conditions to which the parents have been subjected. These facts open up a series of problems of physiological genetics to be studied on locusts. The eventual importance of such studies for a better understanding of locust biology needs no stressing.

In summing up what has been said on ecology, behaviour and physiology, it may be appropriate to stress that locusts and grasshoppers appear to be exceptionally suitable for laboratory studies on a number of fundamental biological problems. It is, therefore, encouraging for acridologists to know that in North America such common grasshopper as *Melanoplus differentialis*, and in Great Britain, some species of locusts are accepted as laboratory animals, and are even likely to replace the cockroach which became one merely because it was readily available. Such a replacement, in my view, would be of advantage to fundamental research, while the results would be of value to acridology and therefore to applied entomology as a contribution to a better understanding of the locust and grasshopper problem.

### POPULATION DYNAMICS

The essential reason why locusts and grasshoppers are important pests is their tendency towards extensive numerical fluctuation. Studies on population dynamics must, of course, be based on reliable quantitative estimates, carried out on populations that remain discrete throughout the period of study, and for any work aiming at deductions of practical value the period must cover several years. The difficulties of such studies are very great. To begin with, there are no very reliable methods of counting field populations, though some, e.g., marking and recapture, provide reasonable theoretical estimates. Also, it is very seldom possible to find a field population that is completely separated from others and remains so for the whole period of investigation. Even when such a population is available, the mobility of Acrididae, including change of habitat and emigration, presents another difficulty. It is not surprising, therefore, that systematic population studies have been very few. Two outstanding examples are a 5-year investigation on a population of British grasshoppers, and work on the Moroccan locust in Cyprus, also for a similar period.

It is of interest to record that, in both cases, weather appeared as the dominant factor in regulating numbers, particularly through its effects on fecundity, but also as a cause of mortality. The regulatory effects of natural enemies, particularly in the case of locusts, are often spectacular, but their importance is reduced by the mobility of the hosts which enables them to escape. Investigations on natural enemies of non-migrating grasshoppers are in progress, particularly in Canada, but numerical estimation of their effects in the field are even more difficult than quantitative studies on the host populations.

Studies on population dynamics, and the factors regulating fluctuations, can produce reliable results only by direct and continuous quantitative work. On the contrary, numerous attempts to approach the problem by a short-cut of establishing broad correlations with climatic factors have not been productive and do not merit encouragement. Even less rewarding have been the endeavours to deduce the relation of plague cycles to sun-spots and similar phenomena.

Since the role of weather in population dynamics appears to be very important, and possibly dominant, all population studies require close cooperation of acridologists with meteorologists. The ecological effects of weather phenomena are dependent on their micro-climatic aspects, the study of which is not a matter for a biologist with an elementary knowledge of the use of meteorological instruments. Micro-climatology itself is a very young science, and its application to ecology is almost entirely in the future.

In a wider field of investigations on seasonal movements of locust swarming populations, work in Africa led to the participation of experts of the World Meteorological Organisation in order to provide the essential synoptic background.

### LAND USE

The general trend in the history of grasshopper plagues is their evident connection with the development of new regions. Their emergence as important pests appears to require several decades from the beginning of exploitation of land, as has been seen in Siberia, North America, South America and Australia. In some cases, as in Siberia and Australia, the connection of plagues with particular systems of land use was established, and elsewhere it can be suggested. The underlying cause is usually some irrational use of land, particularly in conditions that are in themselves favourable for the existence of our insects, such as ecotone zones in semi-arid countries. There is little doubt that most, if not all, grasshopper problems are man-made and new ones are bound to arise in the countries that are only entering a period of intensive development. In the case of locusts, no such direct dependence of plagues on land development is apparent, but evidence is accumulating to show that extensive areas of cultivation close to the areas of locust reproduction tends greatly to increase the risks of wholesale devastation of crops by invading swarms. It is noteworthy that the main threat from *Schistocerca paranensis* in South America and of *Schistocerca gregaria* in Africa and western Asia is in the marginal areas of their respective distribution regions, which are invaded only seasonally. In the case of *S. gregaria*, there is evidence that irrigated cultivation in the normal desert habitat creates favourable conditions for this species.

### CONCLUSIONS

It is my hope that these brief notes may serve the purpose of demonstrating how little is known of those essential aspects of locust and grasshopper biology that make them such outstanding pests. It may be argued—and indeed, this view is popular amongst practical entomologists—that there is hardly any need to learn more about a pest, once we are able to keep it under reasonable control by insecticides. As I stated at the beginning, insecticide control of locusts and grasshoppers is certainly effective, but is a mere palliative. There are no cases on record of a species of locust or grasshopper controlled by insecticides to the point at which such control becomes no longer necessary. The best results are always temporary. Grasshopper control by insecticides in the United States of America has a very long history and is eminently successful in preventing regular crop and pasture losses. However, the grasshopper problem continues to exist there and is likely to demand the continuation of chemical control indefinitely. Another example of successful application of insecticides is seen in South Africa, where the Brown Locust is kept under control by regular operations, but the long-term result appears to be that, although there are no longer vast swarms, the insect tends to become an abundant non-swarming grasshopper, more



difficult to kill. In Africa, the Migratory Locust, *Locusta migratoria migratorioides* R. & F., and the Red Locust, *Nomadacris septemfasciata* Serv., are kept under permanent chemical control in the areas where initial swarms are likely to be formed. This policy of suppressing plagues at the source has proved to be successful, but it must be continued indefinitely, without any relaxation.

The question for acridologists is: should we rest fully content with being able to prevent crop losses by continuous application of palliative measures, or is it our duty to aim at a radical solution of the locust and grasshopper problem? My own view is that these are not alternatives and that a reasonable compromise between the two extreme attitudes can be achieved. It would be a short-sighted and defeatist policy to regard chemical control as providing the final solution of the problem; on the other hand, it would be unrealistic to ignore its unquestionable immediate benefits and merely to hope that the final answer will somehow be found when more knowledge is available. A compromise would be to regard the possibility of containing locusts and grasshoppers within reasonable limits by chemical means as a most needed respite for developing systematic investigations into the causes of the problem itself. Since the problem has undoubtedly been largely the result of human activities, it should be within human power to influence it in the opposite direction. The main obstacle is the lack of the essential knowledge. This knowledge can be accumulated provided we do not rest content with the present partial and temporary successes in killing locusts and grasshoppers as fast as they can multiply, frequently only just averting a disaster. A long-term policy can and should be developed under the cover of short-term measures, which are also in need of further improvements. In this endeavour to get a deeper understanding of the problem, acridologists should enlist the cooperation of other scientists, physiologists, biochemists, meteorologists and geneticists, all of whom will find much of direct interest to them amongst the many and various aspects of our problem. Such a coordinated approach to the problem should, in my view, do much towards dispelling the "darkness and mystery" surrounding it, to which the United States Entomological Commission referred 75 years ago. There is no longer any mystery about it, but many aspects are obscure and will remain so if acridologists rest content with the present purely defensive policy.

## DISCUSSION

ASHLEY B. GURNEY. *Melanoplus rugglesi* of the great plains of North America is an example of a sometimes-migratory grasshopper about which scarcely anything was known when the 1939–1951 outbreak began. Recent field work has disclosed the nature of the solitary phase, and has given the basis for detecting population build-up in what normally are localized colonies. In this case, ecological study has not solved the problem, but it adds greatly to our understanding of conditions, and it permits chemical control to be applied much more efficiently than before.

A. P. ARNASON. Up to now, grasshoppers occur in Canada in economic numbers for some years, and then become insignificant for a time. It is possible to control these outbreaks effectively with chemicals, and they are presumably brought to an end by weather. Is it necessary to devote much effort to ecological investigations under these circumstances?

B. P. UVAROV. Yes, unless it is preferred to continue chemical control indefinitely, at ever-increasing costs, abandoning every hope of achieving a permanent solution of the problem.

J. T. MEDLER. Since grasshoppers in North America are largely associated with crop cultivation, how can we reconcile the need for long-term ecological studies with the continued need for chemical control, which seems to be indicated as long as the crops are cultivated?

B. P. UVAROV. Chemical control offers only temporary solution, and, while practicing it, we should continue seeking the ways of a long-term control which can only be on an ecological basis.

R. H. HANDFORD. Although workers in Canada are pessimistic about finding an ecological control of grasshoppers in the grain crops of the main grasshopper areas, the Canadian Prairies, there is a practical reason for studying fluctuations in populations, namely, a simplification and increased accuracy of forecasts. The aims of such a study would be the same as for ecological control, and the same answers may be found.

# United States Technical Assistance Approach to Problems in Applied Entomology

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## ABSTRACT

*United States technical assistance to foreign countries, as now carried out under the International Cooperation Administration, is based upon the assumption that capital and technical skill when used in a practical way will automatically raise the standard of production and the welfare of the people. Food production consequently has been a major objective in the field of agriculture in most countries. Agronomists and other technicians working in this field have become aware of the tremendous loss to crops and livestock caused by insects, diseases, and rodents.*

*During the past 10 or 12 years entomological activities in foreign technical programs have exerted an interesting effect on the production and protection of agricultural products. Ministries of Agriculture and Departments of Plant Protection in 30 or more countries have cooperated with the United States in seeking more effective weapons and materials to fight their insect enemies. They have entered into agreements providing for equipment, supplies, technicians, and the training of nationals.*

*Twenty-two full-time entomologists now assigned to foreign posts work side by side with local technicians on many phases of applied entomology. Among their many duties, they serve as advisors to the Ministries of Agriculture. They assist in setting up field experiments and demonstrations to test the effectiveness of modern insecticides, the proper use of spraying and dusting equipment and aircraft in large-scale control operations. They conduct training schools for pilots, mechanics, and plant protection personnel. They help develop techniques for assisting quarantine officials and extension specialists.*

*The joint effort of technical assistance organizations in the field of entomology is bringing about a better understanding of the many problems of pest control both at home and abroad. The sharing of knowledge in a practical way has resulted in strengthening plant protection organizations. It has stimulated more interest in pest control and has brought about a spirit of international cooperation that is beneficial to all peoples.*

American interest in technical assistance in the underdeveloped countries of the world is based upon the simple fact that they are vital to our existence. They supply us with approximately 60% of our imports, and consume 45% of our exports. In our defense effort we depend upon them for 70% of our imports of critical strategic materials. Since more than half the people of the world have been living under conditions approaching unbelievable hardships, it was apparent that if we were to continue to depend upon these countries for these supplies something had to be done. Therefore, in January, 1949, we formulated a program for making the benefits of our scientific advances and industrial progress available for their improvement. Positive action to combat poverty, hunger, and disease, in approximately 40 countries got under way.

Eighty percent of this technical-assistance work falls into three categories—food, health, and education. The most urgent of these has been food. Technical-assistance administrators first concentrated on helping people to grow more food. At the same time we were teaching them how to wipe out disease, to establish schools, and to train teachers in an all-out drive to raise living standards. Gradually many changes were introduced, such as improved seed, better preparation of the soil, use of fertilizers, and better use of the land and water.

It was soon recognized that there was need for better protection against insect pests and plant diseases. Changes in agronomic practices often result in conditions favorable to insect multiplication, providing them with means of becoming more menacing. These facts were not overlooked, as better balanced programs were set up in many countries where there had been little or no entomological work. At the request of host countries, entomologists were first sent to Peru, El Salvador, Nicaragua, Ecuador, Costa Rica, the Dominican Republic, Bolivia, and Honduras. Working closely with the local Ministries of Agriculture,



they developed practical programs on the biology and control of their major insect pests. They conducted surveys to determine the species of economic importance and planned demonstrations on the control of insects affecting such crops as coffee, cotton, sugarcane, corn, fruit, and vegetables. Much of their time was devoted to training young men interested in the field of entomology. Most of the actual work has been done by these men side by side with the American technicians. In areas where insecticides were scarce or little known, many of the new highly toxic materials were introduced and field tests made with them under varied climatic conditions. Assistance was also provided in acquainting officials and farmers alike with the use of modern spraying and dusting equipment.

In most of these countries American entomologists are still engaged in these technical assistance programs. Their counterparts, however, are rapidly assuming full responsibility for the entomological work being carried forth. Many of these young men have been sent to the United States for further scientific training in the colleges and experiment stations.

Technical-assistance programs in agriculture in other areas of the world also indicated the need for entomological work in the Philippine Islands, Liberia, Indonesia, Iran, Jordan, Israel, Ethiopia, and Nepal. To each of these countries were sent entomologists qualified to serve as advisors to the Ministries of Agriculture in whatever kind of entomological assistance was requested.

By 1950 and 1951 technical assistance had spread to many countries of the Near East and South Asia. From time immemorial, locust plagues have swept over vast areas extending from Kenya north through North Africa, the Near East, and across to India. In April 1951 the government of Iran requested assistance from the United Kingdom, the U.S.S.R., and the United States, in one of its worst locust invasions on record. The series of events that followed made entomological history. Because of their international importance and their effects in strengthening and promoting better organization of pest control, I believe it worthwhile to discuss their highlights before this Congress. My remarks will therefore be directed on (1) the manner in which the United States has attempted, through its bilateral technical-assistance programs in entomology, to meet the requests from nations of the Near East, South Asia, and Africa, (2) the problems of applied entomology in these areas, (3) the objectives in view, (4) accomplishments during the last five years.

In response to Iran's request for assistance in its locust plague in 1951, the U.S. government rushed spray planes, insecticides, and technicians to the scene of activities. It found a well-organized campaign under way in some of the most difficult and inaccessible areas in that part of the world. In mountainous areas poison bait was being packed by camels and donkeys. Despite all control efforts, there remained sparsely-settled, inaccessible places, which developed infestations that threatened crops. It was here that the Iranians first used the airplanes to advantage, covering large acreages in the short time available and thereby saving many valuable crops.

The enthusiastic cooperation and support received from Iran was very impressive. The emergency aid on locust control clearly demonstrated how modern techniques can be applied successfully in programs of technical cooperation with other governments. The successful operations in Iran resulted in requests from India and Pakistan for the United States to assist them in demonstrational aerial control of locusts during the same year.

Both India and Pakistan, like Iran, possessed well-organized control organizations determined to make use of new techniques, new insecticides, and aircraft in locust control. The small Piper Cub spray plane found its place in the desert. It has repeatedly demonstrated its usefulness in rugged terrain where no ground equipment could be used.

These rather meager efforts in attempting to share our methods and materials proved invaluable for developing other cooperative programs in pest control.

The Regional Insect Control Project, established in July 1954 by agreement between the International Cooperation Administration and the U.S. Department of Agriculture, now serves the U.S. Operations Missions in Lebanon, Iran, Iraq, Pakistan, Libya, Egypt, and Afghanistan. Under the terms of this agreement the Agricultural Research Service of the U.S. Department of Agriculture has delegated to the Plant Pest Control Branch the responsibility for the technical direction of insect-control programs that have been approved by the International Cooperation Administration as a part of the technical cooperation with countries in the Near East, South Asia, and Africa. Field headquarters for the operation of

this project are at Beirut, Lebanon. A field force of nine entomologists and five pilots compose the present staff operating in seven countries.

The work on locust control clearly demonstrates the principles under which technical assistance operates—that capital and technical skill can be used to help raise the standard of production and the welfare of the people. The lessons learned have been advantageous both to the cooperating countries and to the United States. The sharing of scientific knowledge and know-how have not moved in one direction in the form of a “give-away” program. All countries with which we have cooperated on insect control have expended more money and utilized more manpower, equipment, and materials than we have.

During the few years when insect-control work was limited almost entirely to locusts, demands upon our personnel for assistance on other major crop pests became greater and greater. Plant protection officials gained confidence in what we were doing. They had learned more about our motivation and the nature of our approach to problems that, for all practical purposes, were common to us all. They were eager to reappraise and improve their pest control organizations and appropriated larger funds for plant protection. Consequently, in such countries as Iran, Pakistan, India, and Iraq we have practically withdrawn our participation in desert locust control. These countries now have their own aerial units with trained pilots to conduct their spraying operations, and their locust infestations have been reduced to a minimum.

Our operations under the Regional Insect Control Project consist largely of control demonstrations on major insect pests affecting food and fiber crops. Briefly, our objectives are (1) to assist the United States Operations Missions in their efforts to aid the Governments of cooperating countries in the development of practical control programs and supervise and direct such segments of these programs as may be agreed upon, (2) to maintain facilities and continue needed services in assisting these countries in the control of the desert locust through the demonstration of aerial spraying techniques and reconnaissance as a supplement to other control measures, (3) to train local pilots and mechanics to provide for the continuation of aerial spraying and to train local entomologists in field survey and control methods, (4) to give guidance and assistance in the coordination and execution of aerial and ground control activities, (5) to promote international cooperation by working with technicians and authorities of individual nations and agencies concerned with the control of the locust and other major pests.

The work in each country is based upon an agreement drawn up by representatives of the Ministry of Agriculture in that country, the local International Cooperation Administration Mission, and the coordinator of the regional project. Bilateral agreements are for one year, but are extended or amended according to the needs and availability of funds. Each agreement provides that a host country furnish (1) ground support for aerial spraying, gas and oil for the aircraft, and assistance in its maintenance, (2) transportation of insecticides, fuel, water, and other supplies, and (3) technicians to supervise their own country operations and cooperate with the regional control personnel.

In 1952 under the original locust control program nine Piper Cub airplanes, equipped with the necessary instruments, spraying gear, and spare parts, were purchased and placed at the disposal of the coordinator. They were operated by pilots and mechanics provided for under a commercial contract. The pilots worked in units of two or three, with an entomologist supervisor over each unit. Ten planes are now distributed throughout the area. Five pilots are still under contract for aerial application of pesticides, maintenance of the planes, and training nationals.

In addition to the planes, pilots, and entomologists, the United States contributes a part of the insecticides used in demonstrational control work, the equipment, and other miscellaneous materials necessary to carry out the project.

Much time and effort have been required to stabilize this work and get it running smoothly. Although the initial phase of the project is over, additional time and continued efforts are necessary to establish the things that have been started so that there will be less chance for their retrogression. The adoption of new methods and new skills does not happen overnight. Neither do our own technicians find that all they have to learn comes easily. They must adjust to differences in customs, personalities, government, and administrative policies, often through the medium of a strange language. Most of the insect species are new to them. They must be flexible enough to pass through the frustrations resulting from lack of

accomplishment or delays, as well as those arising from certain cultural and psychological disparities between themselves and their foreign colleagues. But these things may work both ways, and be as common to the foreign technician. He too often finds it difficult to understand us. There must be patience, tolerance, and willingness on both sides, and a common meeting ground if we are to understand each other and adapt to new situations.

In selecting personnel to represent the United States in this program, we must be sure that they meet all the requirements called for. Besides having scientific training and practical experience in insect control, the individual must be stable and well-informed, capable of independent judgment, and have initiative. He must be cooperative, friendly, and willing to work under adverse conditions where great distances make for poor communications and supervision is at a minimum.

The duties of our overseas entomologists include (1) survey and evaluation of infestations and control needs, (2) aid to the U.S. Operations Missions in planning practical control programs, (3) technical service to the Mission experiment stations and extension services, (4) advice to the chiefs of plant protection in the Ministries of Agriculture, aiding them in organization of control programs, (5) demonstration of new insecticides and equipment, (6) training of nationals in entomological control methods, and (7) aid to the Missions in selecting personnel for training in the United States.

Our aim in carrying out these programs is to build stronger and more capable control organizations within countries by encouraging them to assume the major responsibility. This is true not only in locust control but also in that of other serious pests such as the pyrrilla of sugarcane, the senn pest of wheat, spiny bollworm of cotton, rice stem borer, and olive fly. Plant protection officials are no longer content with the antiquated control methods of the past. Even though they are short of trained personnel and facilities for research and control, our greatest hope rests in the fact that they are doing something to better the situation.

We are now in our sixth year of working side by side with the plant protection technicians. During this period we have observed many changes. Through better country organization we have experienced more effective international cooperation. This is quite evident in locust control. Much credit should be given the Food and Agriculture Organization of the United Nations, which has been influential in promoting and coordinating combined international action.

It is difficult to judge our total accomplishments during the short period this work has been in progress. Some measure of influence might be gained from the following figures: Our records show that entomologists of the Regional Insect Control Project have been called upon to perform 327 demonstrations on the control of 75 insect species in 10 countries. Eleven kinds of insecticides have been used on 32 different crops on a total of nearly 367,000 acres. The host countries imported 400 tons of modern insecticides, 49 spray planes, 188 trucks, 544 power sprayers, and nearly 11,000 hand sprayers. Our field personnel have trained 40 pilots and 24 airplane mechanics to operate and maintain spray planes, as well as 142 plant-protection officers. There has been a steady increase in the number of entomology students who have come to the United States for training in research, control, quarantine, and extension work. Nineteen have come from the Near East in the last five years.

The fine spirit of teamwork in this bit of international cooperation has left its mark on farm folk all the way from Lebanon to India. It will continue to make an even greater impression as these people find permanent solutions to some of their problems. We are proud of what has been accomplished so far. We are also confident that this progress will continue as more technicians are trained and provided with the necessary support to carry on the everlasting war against our insect enemies.

## DISCUSSION

ALDEN D. HINCKLEY. What is the recruitment system used in your program?

EDSON J. HAMBLETON. We have recruited largely from our own personnel within the Plant Pest Control Branch of the Department. When not available we must seek qualified men from other sources. Recruitment for this program is conducted through established procedures in the Department with the concurrence and approval by the Int. Coop. Administration.

# Applied Entomological Activities in the United States

By M. P. JONES

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## ABSTRACT

*This talk is based on information usually given to entomologists from abroad, who visit our offices, and who want to know how we get public participation in the application of insect control measures.*

*In the United States, there are several thousand entomologists working in the fields of research, quarantine, education, control, sales, and service. This paper will relate to those in public service. The entomologists in quarantine, work to prevent introduction and spread of insect pests. Some of those in research are developing more effective and economical control measures and methods of applying insecticides. Another group works on cooperative Federal-State eradication and control projects. Some make surveys of the presence and abundance of insects. Entomologists in the above groups, as well as those in industry, assist in the educational phase of entomology. In addition, there is an Agricultural Extension Service that is charged with carrying on an organized educational program in subjects relating to agriculture and home economics.*

*One phase of this over-all program is Extension entomology. These entomologists expedite and guide the application of the most effective and economical insect control measures. They work through some 2,880 State Extension workers and 11,100 County Extension workers trained in the broad field of agriculture and home economics.*

*The 80 State Extension entomologists prepare and distribute bulletins, circulars, press releases, service letters, and visual materials. They also present their message by radio and television, at public gatherings and in personal contacts by letter, telephone, office calls, and farm visits. Demonstrations relating to pest control, set up by County Extension agents, are also supervised by these men.*

*Entomology in the United States is big business. Its effects are far-reaching and influence the lives of practically all people, rural and urban. Some 4,500 professional entomologists and possibly a greater number of nonprofessionals earn their living by working on various entomological problems. The salaries of these people plus the cost of insecticides and other expenses relating to their manufacture and application are estimated to be about one billion dollars annually*

*The job is not complete, however, because despite these costs, insects continue to ravage crops, livestock, and property worth about \$3,600,000,000 annually.*

*This topic is so broad and inclusive that some definition of the field to be covered should be given. Applied entomological activities as herein discussed refer primarily to field aspects of insect control as contrasted with the fundamental or academic phases.*

*Reference to research work will appear throughout the sessions at this Congress; therefore, further reference to it will not be made in this paper, except to state that the importance of all phases of research is fully recognized, and that the results of research are the basis for the educational and control work.*

*The speaker's official responsibility is in the field of Extension entomology. He will, however, cover briefly the fields of quarantine, control and regulatory functions, and Extension.*

*Entomologists engaged in Federal plant quarantine work enforce quarantines and regulations designed to prevent the entry into, and subsequent spread within, the United States of injurious plant pests. These men are located at major air, sea, and border ports of entry in the continental United States, Hawaii, Puerto Rico, and the American Virgin Islands. They inspect incoming aircraft, ships, automobiles, trains, and other means of conveyance, as well as cargoes of plant material. They also examine parcel post for the presence of plant material that might carry harmful pests. In cooperation with the Customs Service, they examine the baggage of passengers from abroad for the same purpose. They prescribe and supervise fumigation or other treatment of commodities that require such safeguarding as a condition of entry. Plants that might carry diseases not detectable at*



the time of entry are required to be grown under post-entry quarantine until determined by periodic inspections to be pest free. Other plant products, such as bulbs, may be inspected prior to shipment in foreign countries by United States inspectors and certain products may be treated at approved stations in foreign countries under the supervision of United States inspectors before shipment to this country. The inspectors enforce similar restrictions on plants and plant products moving out of areas of the United States that have been quarantined to prevent the spread of specific pests in this country.

Many entomologists are also employed by state regulatory organizations to enforce state plant quarantines and nursery inspection requirements. All states require nursery inspection certificates for movement of nursery stock from other states and many have quarantines governing the entry of plant material from other states in interstate commerce.

The Plant Pest Control Branch, Agricultural Research Service, in cooperation with states, industries, and local agencies carries on pest control and regulatory programs in various sections of the United States, where the pests involved are a serious hazard to field and forest crops. The programs are jointly planned and executed with cooperating agencies, and carried out on a community or areawide basis. Control and regulatory operations are based on the results of research by entomologists in the U.S. Department of Agriculture and the State Experiment Stations. The work of these programs consists of surveys, field testing and demonstration of the results of associated research, application of control and eradication measures, administration of domestic plant quarantines, coordination and technical direction of field operations, and stimulation of cooperative effort among the agencies concerned.

Various kinds of surveys are carried on in connection with the operation of insect pest control programs. They include surveys to determine the distribution of the pests, to delimit areas of infestation, and to locate host plants. Still other types of surveys check the effectiveness of control measures and determine the compliance with regulations affecting the control of the pest. The object of these surveys is to obtain information necessary for the efficient conduct of control operations.

The field application of control measures by this branch involves many types of activity, for example, spraying, dusting, soil treatments, sterilization of infested crops, and fumigation of stored products. Such measures may also include roguing of host plants, clean culture, production and release of parasites, and enforcement of quarantines and pest control regulations. The application of control measures requires the use of a wide range of equipment. Airplanes, power sprayers and dusters are frequently used along with hand applicators, sterilization and fumigation equipment, specially adapted farm equipment for soil treatments, and hand tools and labor for eradicating host plants.

Control programs are planned and operated in close cooperation with states, counties, townships, communities, and industry. Cooperating agencies are given technical assistance and encouraged to participate to the fullest extent possible in work projects. This is accomplished through personal interviews, joint planning, publications, radio, television, newspapers, and other channels of information.

State agricultural agencies enforce state quarantine and pest control regulations, and cooperate in the control of insect pests affecting field and forest crops.

The remainder of this paper relates to insect control through education. The remarks are based pretty largely on questions so often asked of Extension entomologists by visitors from foreign lands.

The Extension Service is often referred to as the educational arm of the Department of Agriculture. It is even more than that, however, because it serves as the primary off-campus educational arm of the land-grant colleges in the 48 states and 3 territories. It is the largest adult educational organization in existence. More than 14,000 professionally trained men and women in all phases of agriculture and home economics make up the field force of the Extension Service. Approximately 11,000 of these are located in over 3,000 counties as men and women county Extension agents. Over 3,000 are in the state offices and about a hundred in the Federal office. About 85 men of the state office personnel are entomologists.

While it is the largest, the Extension Service is not the only educational agency servicing the American public. Much assistance is given by colleges, experiment stations, other state and Federal agencies, and commercial companies.



Extension Service in the United States was created to fulfill the desire of farmers to have greater and more intimate counsel on their farm problems. After the agricultural experiment stations began organizing in 1877, crop and livestock production research was developing faster than farmers were getting the information. As a result, county, state, and Federal governments began providing liaison service. This took the form of short courses, farmer's institutes, correspondence courses, professors' appearances on programs at agricultural meetings and many other forms. Later county agents were employed and State Extension services were organized. As more technical information was developed by experiment stations and the U.S. Department of Agriculture, there arose a need for more technically trained personnel on the State Extension staff. Consequently, subject matter specialists were employed. The first specialists were in the broader fields of agronomy, horticulture, and animal husbandry, gradually working into the more highly specialized fields. The first Extension Entomologist, Mr. T. H. Parks, was appointed in Idaho, April 1, 1913, to help combat the alfalfa weevil threat. This field has continued to expand. At the present time, Extension Services in 46 States and Alaska employ 85 entomologists, including 9 States which employ 9 apiculturists.

In 1914, the Smith Lever Act was passed by the Federal Congress, and became a public law. It provides for Federal-State Cooperative Extension Work with county, state, and Federal financing and administration. In the fiscal year of 1956, 113½ million dollars was available to the Cooperative Extension Service. About 24 percent of this came from within the counties, 33 percent from state and 43 percent from Federal sources. The expenditures of these funds presents a different story. About 68 percent was spent in the counties, 31 percent in the state offices, and less than 2 percent in the Federal office. Of that spent in the state offices, about \$700,000 was spent for salaries and expenses of Extension entomologists.

Much strength of the Extension educational program is in the work of about a million voluntary men and women, who serve as local leaders. This group of loyal people supports the Extension program by conducting demonstrations, serving on committees, relaying information, keeping records, and helping to organize local discussions or work groups.

Now, that we have a picture of Extension organization and financial arrangements; how is it used to assist the public with entomological problems?

The county Extension agents are the key men in carrying out educational information on insect control. Therefore, the first responsibility of a State Extension Entomologist is to keep county Extension workers currently informed. Such information, not only involves subject matter, but methods for disseminating it. County agents and other Extension personnel also work in close cooperation with Plant Pest Control officials.

A knowledge of the insect pests likely to be encountered in a county and state during the ensuing year is one of the first needs in assisting agents. This information is obtained from past experience, from surveys made by the Extension entomologists, by special survey men, and other entomologists. County agents also learn much about the insect problems in their counties during their day by day contacts with rural and urban people. Most of the technical information they use, however, comes from their subject matter specialists.

The Extension entomologist gets his information from a number of sources, but primarily from his own state experiment station. Professional journals, bulletins, and leaflets from other States and the Federal Government are used extensively. Another source is gathered from subject matter discussed at national and regional conferences. Some of the most productive regional conferences are those where entomologists in research, extension, and control meet to discuss the results of research and field experience, after which over-all recommendations are formulated. Representatives of the Food and Drug Administration under the Department of Health, Education, and Welfare, and of the Pesticide Regulation Section under the U.S. Department of Agriculture are often present. The contribution they make about their functions and actions is especially valuable at the present time. The over-all recommendations are later reviewed by the entomologists in each state in light of their research and local situations. By this procedure, states having a common problem are able to draft recommendations that are as uniform as possible. Regional conferences of this nature have been held on such subjects as: cotton insects, forage insects, fruit and vegetable insects, livestock insects, stored grain insects, and grasshoppers.

With a knowledge of the possible insect pests and the results of experiments on insect control at hand, bulletins, and leaflets are prepared, usually in cooperation with research entomologists and sometimes with specialists in related fields. Some bulletins are general and give information about a wide range of insects. Those designed as handbooks for county agents and other leaders often include references to the kinds of insecticides to use, the dosage rate, the time of application, and the number of applications. They may also state the time necessary between the last application and harvest, and possibly the residue tolerance allowable by Food and Drug Administration officials. Many publications for distribution to the public cover recommendations for a specific insect, a group of related insects, or the insects of a particular crop. Such publications usually give more information about the life history, habits, and control measures for the particular pests.

Aside from bulletins, county Extension agents are kept currently informed by weekly insect news notes or service letters. Such letters carry information about the relative abundance of the insect pests and beneficial insects, also about any new recommendations or shifts in those made earlier. In some states, county agents contribute items for the news notes.

County Extension workers are also fortified with information to meet local situations during special discussion conferences or workshops arranged either on a district basis for small groups of agents, or on a statewide basis. Specialists in the related fields of entomology, plant pathology, agronomy, horticulture, animal husbandry, or agricultural engineering, often participate in such conferences. Such workshops aid the agents to better understand the many facets of the problem concerned with the production of food, feed, and fiber.

Farmers are also assisted when county agents alone or in company with the entomologists conduct public meetings. Some such meetings are held during the off-crop season, where background information is presented. Lantern slides, charts, and motion pictures, as well as exhibits, are often used to illustrate the subject. Other meetings and demonstrations are held in the fields during the growing season, where farmers are taught to recognize the insect pests and beneficial species, how to make sweepings, or make "counts" of insect populations, how to determine the proper time to apply insecticides, and how to properly adjust spray and dust equipment to meet the particular need.

Daily and weekly newspapers, farm magazines, radio, and television are used extensively for the dissemination of pertinent agricultural news. These mass media for education reach large numbers of people. They are useful in alerting farmers to situations, for announcing and giving reports of meetings, and to disseminate a certain amount of subject matter information. Every effort is made to have farmers keep insect control measures in balance with his total farm operations. That is with rates of seeding, fertilization, irrigation, and in some instances market demands.

An intensive program is under way to teach the youth in the United States more about the importance of entomology. About 28,000 members are enrolled. One incentive in this work is the National 4-H Entomology Award Program sponsored by the Hercules Powder Company. It provides for county, state, and national awards. The project affords an opportunity for 4-H Club members to make collections of insects, to conduct life history studies, to do insect control work, and to give demonstrations on entomological practices.

The Extension Services in 9 states employ apiculture specialists. In other states the work in beekeeping is carried by the regular Extension Entomologist along with his insect control work. Apicultural information is handled through county Extension agents and in much the same way as that outlined above for general entomology.

The Federal Extension Entomologist serves in a liaison capacity between research entomologists in the Department and the Federal Extension Service, between the various Federal offices and the entomologists in the State Extension Services and between the different State Extension Services. Related specialists in the states are kept informed of research developed in the Department of Agriculture and other Federal agencies. They are also informed of the actions taken by the Federal Food and Drug Administration and the Pesticide Regulation Section, Agricultural Research Service. Such actions relate to insecticide registrations under the Federal Insecticide, Fungicide, and Rodenticide Act of 1947, and to residue tolerances established under the amendment to the Food and Drug Act, Public Law 518, commonly referred to as the Miller Bill.

Through an organized exchange of publications, issued by the State Extension Services and the entomologists in the Department, each group has the benefit of the recommendations of the others and the kind of publications being issued.

The full impact of Extension's educational program can be realized when we know that during 1955 farmers were assisted with insect control and beekeeping problems in 7½ million instances, according to county Extension agents' reports. Several surveys have been made to determine the rating of questions and problems relating to entomology as compared with other phases of agricultural production and conservation. One such survey revealed that 17 percent of all questions asked of county Extension agents relate to insect control—the percent increased to 35 percent during the crop growing season. In another survey, a county agent in one of the Southern States sent a list of some 30 agriculture problems to the farmers in his county, asking them to rate the problems in order of their importance—1,924 farmers, or 10 percent, rated insect control as the most important.

The American public has a greater appreciation of the value of insect control than in any previous period in history. This fact, coupled with the wide range of insecticides presently in use and the new Federal legislation relating to the sale and the use of pesticides, has changed the nature of the Extension program. It is no longer necessary to demonstrate to farmers the value of insect control. They now want to know what insecticide to use, and how and when to apply it. This has necessitated the development of an educational program to help the public to better recognize their insect pests and to know when an insect population is sufficient to warrant applying control measures. To arrive at this conclusion, various situations are taken into account, for example, the potential crop yield, the presence and abundance of beneficial insects or diseases, the weather, and the period of time until harvest.

Educational or service programs of this type are conducted under different names, such as supervised control or scouting programs. One example of this kind of program is cotton insect scouting in Arkansas. It started about 1923, but the greatest expansion has been in the last five years. Since the beginning, it has consisted of the regular scouting of certain cotton fields. The procedure in 1955 was somewhat as follows: Growers paid a per acre fee, or monthly salary, to have scouts examine their cotton fields once each week, or oftener, throughout the active insect season. The primary function was to determine the presence and abundance of insects; however, when they are asked for counsel on control measures, such information is given. Operators of large acreages employed several scouts. Growers of small acreages banded together to employ one scout. Each scout examined from 1,000 to 1,400 acres weekly. Sometimes they found it advantageous to work in teams of two men. About 90 scouts were employed in the state, and they examined 140,000 acres or 10 percent of the state's cotton acreage. The salaries and expenses of these scouts, who were under the supervision of the Extension Service, were paid by the university from funds paid to the university treasury by growers, whose fields were examined. Some scouting in Arkansas was on a different basis. For instance, bankers and cotton ginneries provided scouting service for their patrons on a reimbursable basis, but all cooperated closely with the Extension Service. Most of the scouts were college students, who had had courses in entomology. They were given special training at the university and in the field just prior to starting to examine cotton for the farmers. The close supervision throughout the summer by the Extension Entomologist and the survey entomologists afforded the best possible service to participating farmers.

Cotton growers, who had employed men to examine their fields in previous years, stated that "the greatest satisfaction was in the peace of mind they got from knowing that their cotton was being examined for insects regularly." They have also been able to better time the applications of insecticides, and in some instances omit one or two; thereby, obtaining better insect control at a saving. An interesting and wholesome situation resulted from the paid scouting program. Some of the farmers, who followed the scouts into their fields, discovered that they could check their own cotton, and in recent years have been doing so.

Scouting to determine insect populations as a means of knowing the need or time for applying insecticides, has been used with a number of crops. There is every reason to believe that this type of service will continue to expand. In fact, some entomologists predict that some day specialists will diagnose troubles and prescribe treatments for insect pests, much as is done in the veterinary and medical profession. The complexity of the problem,

the possible residue hazard, and the specificity of insecticides may bring this about. It is doubtful, however, if such a service will ever replace the existing Extension, control, and regulatory programs.

Even though entomology in the United States is recognized as an essential activity and a necessary adjunct to a well-rounded agricultural enterprise, it is not the intent of the writer to present the foregoing program as the ideal for all countries to follow. Local situations will necessitate variations. We wish to re-emphasize, however, that Federal and state agencies, as well as industry, are working together to prevent the introduction of new pests, the spread of localized ones, and to help the American farmer apply the most effective and economical control measures.

We are grateful, therefore, for the opportunity of presenting this paper on Applied Entomological Activities in the United States at this, the Tenth International Congress of Entomology, and sincerely hope that we have contributed some information of value to entomology in other countries.



# Advisory Entomology in England and Wales

By F. H. JACOB

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In England and Wales research, advice and regulatory work in Applied Agricultural Entomology are administered in different ways. With few exceptions research is the responsibility of the Agricultural Research Council (A.R.C.) appointed by the Privy Council. Advisory or Extension Entomology is provided by the National Agricultural Advisory Service (N.A.A.S.) in the Education and Advisory Services Division of the Ministry of Agriculture, Fisheries and Food. The administration of regulatory work is undertaken by the Horticulture Division of the Ministry. The Plant Pathology Laboratory acts as a liaison centre between the research and advisory sides, and provides the scientific basis for plant health work. Though not a part of the N.A.A.S., it works in close harmony with the entomologists of that service.

For advisory purposes the country is divided into twelve Provinces and Sub-provinces each with a staff of entomologists responsible for local advisory work and investigation. Altogether there are 70-80 Advisory Entomologists.

Another body, the Agricultural Improvement Council (A.I.C.) is directly responsible to the Minister. Its primary duty is to review the progress of research and the needs of the industry in order to advise how best to raise the technical standards of production by means of development or other work. Close liaison is maintained by a joint A.R.C./A.I.C. committee. The Secretary of the A.I.C. is also Secretary of the joint committee, and is one of three senior Education and Advisory Officers of the N.A.A.S. having responsibility for all the science groups (entomologists, plant pathologists, bacteriologists, and chemists).

The easiest way to explain and to understand this organisation, is by means of diagrams.

Fig. 1 illustrates the relationships of the research, advisory, and regulatory facets of the service. Unbroken lines represent direct administrative relationships and broken lines, scientific liaison.

Fig. 2 shows how the advisory service is organised, unbroken lines illustrating the direct links with headquarters and broken ones the close liaison at all levels.

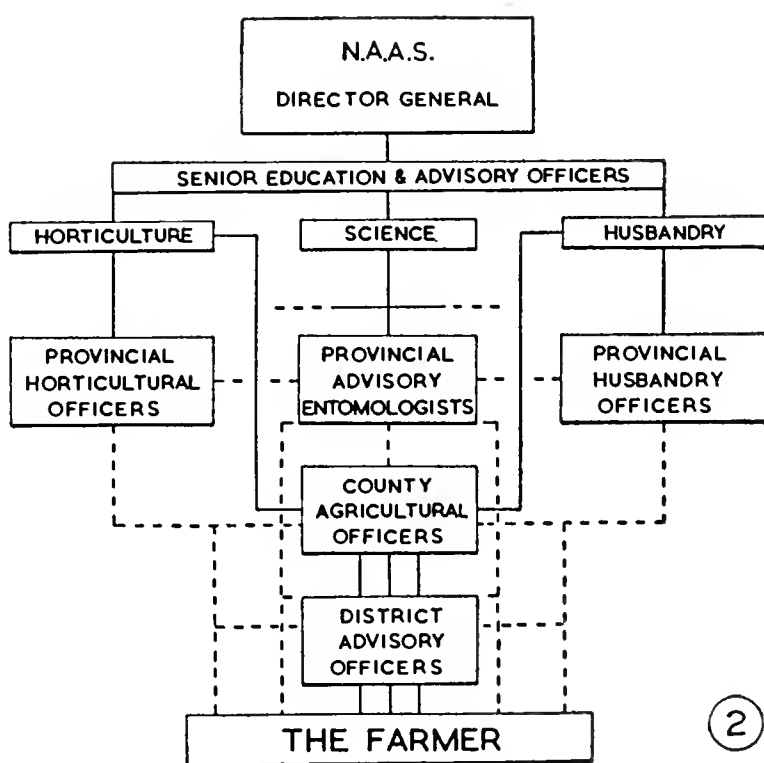
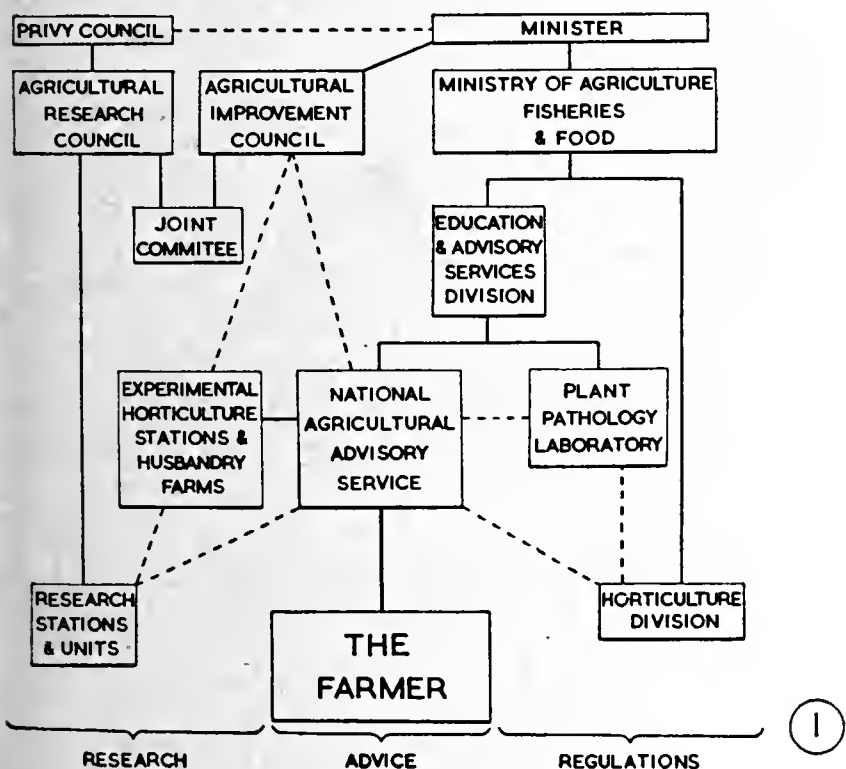


Fig. 1. The inter-relationship of research, advisory, and regulatory services. Fig. 2. The organisation of the National Agricultural Advisory Service.

Fig. 3 shows the boundaries of the eight provinces and four sub-provinces into which England and Wales are divided. It also shows the location of the provincial centres and Experimental Husbandry Farms and Horticulture Stations. Each province includes a number of counties, each with its County Agricultural Officer responsible for all advisory work



The image contains two maps of the United Kingdom. The left map, titled 'N.A.A.S. PROVINCES', shows the division of the country into eight provinces: Northern, Yorks & Lancs, East Midlands, West Midlands, Wales, South Western, South Eastern, and Eastern. Provincial centres are marked with solid circles (e.g., Newcastle, Leeds, Birmingham, Cardiff, London), and sub-centres are marked with solid triangles (e.g., Bangor, Abertystwyth, Reading, Norwich). The right map, titled 'N.A.A.S. EXPERIMENTAL FARMS & STATIONS', shows the locations of various experimental farms and stations across the country. Husbandry farms are marked with solid circles (e.g., High Mowthorpe, Gleadthorpe, Boxworth), horticulture stations with solid triangles (e.g., Fairfield, Stockbridge, Kirtton), and demonstration or sub-stations with open triangles (e.g., Blyn Adol, Trawscold, Cleppa Park, Wisley, Brogdale, Elyforda, Rosewarne). Other locations marked with solid circles include Great House, Bognor, Rosemaun, Allington, Liscombe, Ellbridge, and Starchess.

Fig. 3. National Agricultural Advisory Service Provinces, Provincial Headquarters, and Experimental Centres.

# Training Activities Related to the Control of Insect-Borne Diseases in the United States

By HARRY D. PRATT  
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## ABSTRACT

*During the past 15 years the Communicable Disease Center, or its predecessor, the Office of Malaria Control in War Areas, has played a key role in controlling two major insect-borne diseases, malaria and murine typhus. The problems encountered in controlling both diseases have been attacked by a team of entomologists, engineers, and epidemiologists.*

*Data are presented showing the reduction in malaria from over 100,000 cases and 4,000 deaths a year in the early 1930's to only ten primary indigenous cases in 1955, and of murine typhus from some 5,400 cases in 1944 to 131 in 1955. Brief mention is also made of the problems and programs to control encephalitis, diarrhea, and dysentery now being carried out by the Communicable Disease Center.*

*A training program was developed for the instruction of key personnel at the local, state, and federal levels. This teaching included identification of vectors of malaria and typhus and the best methods of controlling these insects.*

*Many new teaching aids such as specialized manuals, pictorial keys, filmstrips and motion pictures were developed by the Communicable Disease Center to aid in this training program. During World War II the Federal Government furnished a major portion of the money for personnel, equipment, and materials. As the threat from malaria and murine typhus declined, the state and local health departments took over an increasing proportion of these control programs. The training of vector control personnel has been carried out to the states through six regional field training stations and by a series of decentralized courses. This instruction has been broadened to include the control of many types of insects of public health importance such as flies, fleas, ticks and lice.*

*I am very happy to participate in this symposium because of the close relationship of economic entomology and medical entomology as practiced by the Public Health Service. Our Communicable Disease Center has played a prominent part in controlling two important insect-borne diseases, malaria and murine typhus, in the United States and is engaged at the present time in extensive research and demonstration programs dealing with many insect-borne diseases such as encephalitis and diarrhea and dysentery.*

*Our organization has attacked all of these diseases through the team approach, utilizing the combined talents of epidemiologists, entomologists, and engineers. Let us consider first the program element of this symposium, with two important diseases: malaria and murine typhus.*

## MALARIA

*Malaria was once the most important insect-borne disease in the United States. In the period 1933 to 1937, over 100,000 cases of malaria were officially reported. As a result of an extensive public works program with emphasis on drainage, larviciding, and ditching, the number of cases gradually dropped to about 60,000 by 1942, when this country entered World War II. Shortly thereafter, the Public Health Service established an Office of Malaria Control in War Areas with the primary purpose of controlling malaria around military installations and in key civilian areas. There was a slight rise in incidence in 1945 which was due to infected veterans returning from malaria areas overseas. Late in 1945, DDT was made available for an extensive residual spray operation, particularly in the southeastern United States.*

*On July 1, 1946, our organization became the Communicable Disease Center with the Extended Malaria Control Program as one of its major operations. Literally millions of DDT residual applications were made by the cooperative CDC-State health department teams. Malaria dropped from some 60,000 cases in 1945 to approximately 2,000 in 1950. An increase of cases in 1951 and 1952, attributed to returning veterans from the Korean conflict, was followed by another sharp decrease which has continued to the present time.*

In 1955 there were 478 cases of malaria, of which only 10 are believed to be new, primary indigenous cases.

### MURINE TYPHUS

The research work of Maxcy, Dyer, and other Public Health Service personnel during the period 1925 through 1940 showed that murine typhus was a rickettsial disease, widely distributed in the southeastern United States, and that it was transmitted from a domestic rat reservoir to man by the oriental rat flea. The number of cases of murine typhus increased markedly after 1940, as shown in Chart II, and reached a peak of 5,401 reported cases in 1944. Research work in 1944 demonstrated that DDT dust would control oriental rat fleas in San Antonio, Texas (Davis, 1947) and Savannah, Georgia, (Ludwig and Nicholson, 1947) and reduce the incidence of murine typhus in humans. An extensive murine typhus control program, inaugurated late in 1945, was based largely on dusting rat-infested premises to control the oriental rat flea. The success of this program may be judged by careful study of Chart II which shows that the number of cases of this disease decreased in the eight-year period from 5,401 in 1944 to 200 during the period from 1952 to 1955.

### TRAINING ACTIVITIES

Programs of these types are dependent on high caliber, well trained personnel. Since a large number of people with experience in malaria control had joined the Armed Forces of the United States, a training program was set up soon after the Office of Malaria Control in War Areas was initiated. This program, which helped train the entomologists, engineers, and epidemiologists who would direct and coordinate the malaria and typhus control campaigns, was continued as our organization worked on other insect-borne disease control programs such as yellow fever, diarrhea and dysentery, and encephalitis. Basically, these training courses combined classroom lectures and laboratory sessions with a large amount of practical field work. Initially, instruction was based on the experience of other organizations, such as state health departments and other government agencies, with malaria control drainage or paris green dusting. As changes in control practices evolved, the training program became based more and more on research carried out and field tested by our own organization. For example, the operational manuals on DDT residual spraying of houses or mosquito larviciding with DDT were based largely on experience of our research field men at the Technical Development Laboratories near Savannah, Georgia. A small "Hollywood" was set up staffed by artists, photographers, and script writers who worked in close collaboration with the field and research men. A whole new series of motion pictures, filmstrips, slides, pictorial keys and manuals were developed which could be used at CDC headquarters or in decentralized classes throughout the country. A pattern of activities has gradually evolved which has been followed with each successive program: first, field investigations and basic research; second, demonstration projects where control practices are tested on a large scale; third, training schools and consultation with state or local health departments; and fourth, a full-scale control program.

In a Symposium on Extension Entomology such as this, one of the most important questions is "What has been the impact of this training program on public health practice in the United States?" During the 10-year period of fiscal years 1947 through 1956, some 4,500 students have attended 234 courses in vector control throughout the United States. One hundred and fifteen of these courses have been held outside Atlanta in 32 States. In addition, some 1,500 sanitarians and sanitary engineers have received from three to five days instruction in vector control in the basic 12-week environmental sanitation courses conducted by the Communicable Disease Center. These courses have been attended primarily by personnel of state, county and local health departments and mosquito control organizations. However, health workers from foreign countries and limited numbers of industrial employees whose work was related to public health have also attended them.

At first the vector control courses, which were inservice training activities, were largely concerned with the control of malaria and murine typhus. As these diseases were brought under control, training courses were redesigned to meet the changing needs and programs of the states. Practical field courses in fly control, mosquito control, and general insect and rodent control were developed, as well as more advanced courses in the biology and identification of arthropods of public health importance. These courses range in length from one to two weeks. Their level varies from basic courses for newly employed personnel

to highly specialized courses in insect identification and vector control. All courses emphasize the practical aspects of control and are designed to fill the gap between academic training and actual field experience.

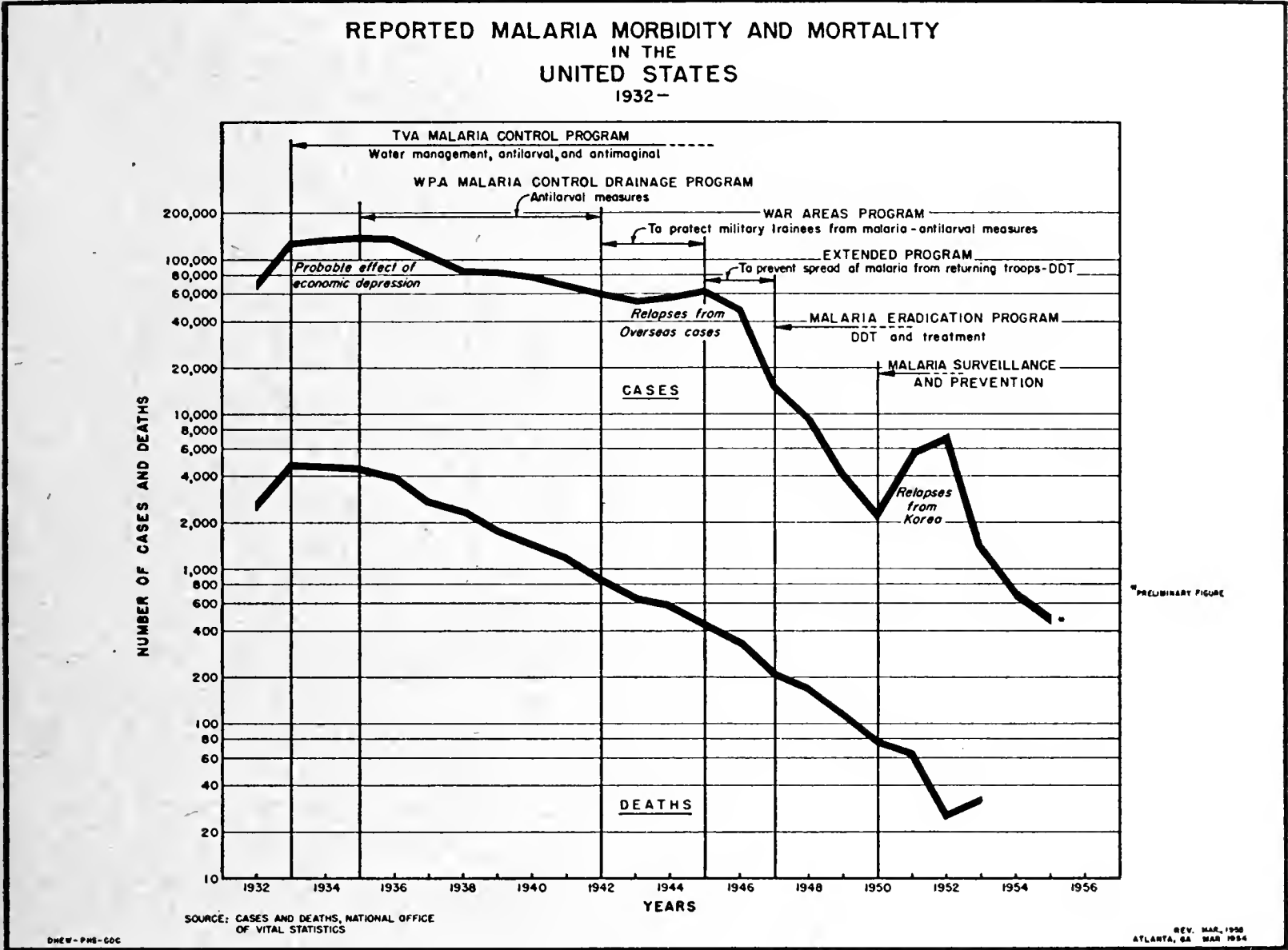


CHART I

**ANNUAL TOTAL OF REPORTED MURINE TYPHUS FEVER CASES  
IN THE UNITED STATES  
1941 - 1955**

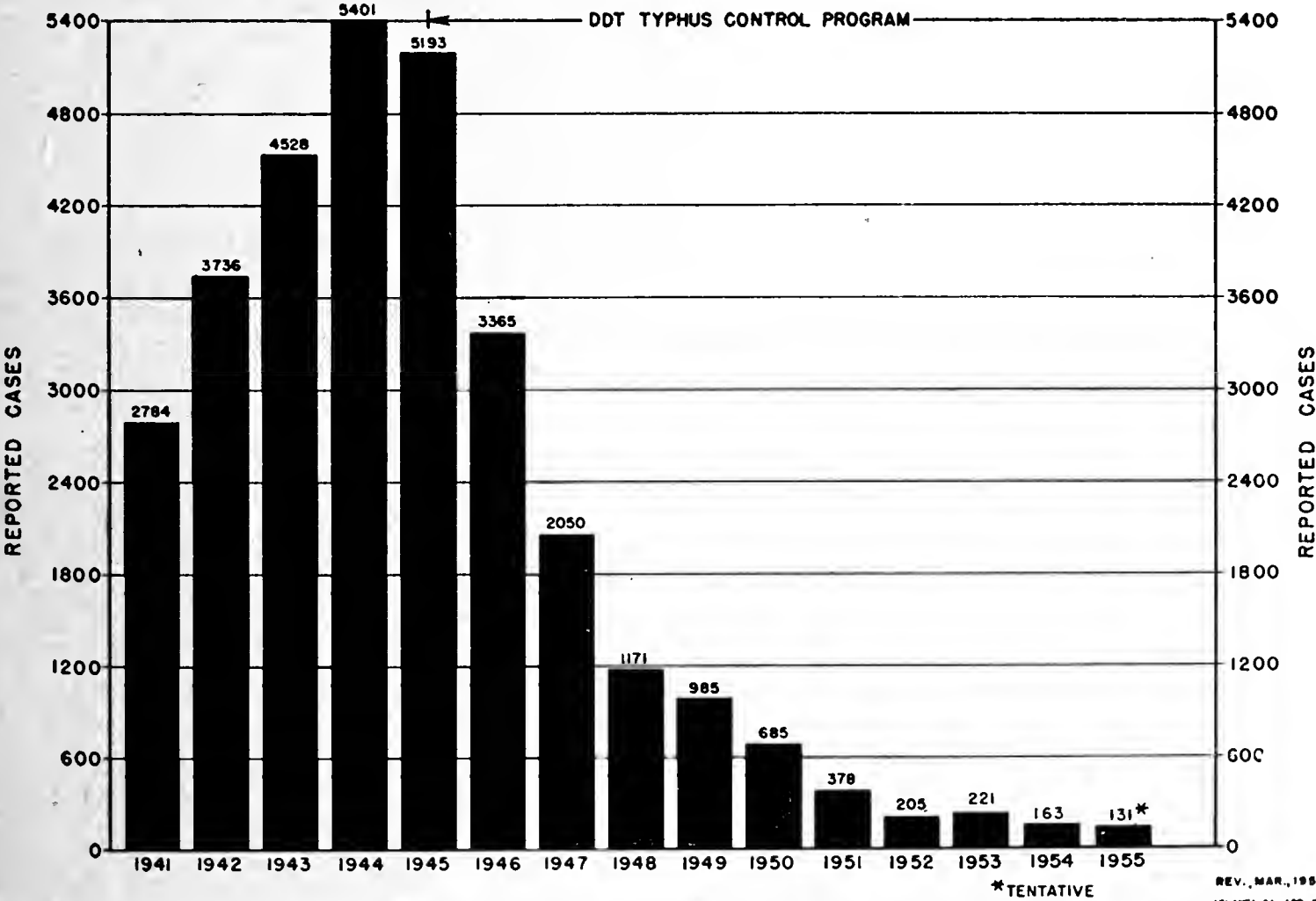


CHART II

The need for such short refresher courses in the United States is very great. The Division of State Merit Systems of the Department of Health, Education, and Welfare has listed the annual turnover of personnel in state health departments in the United States for the five-year period 1950 through 1954 as being between 26 and 29 percent. If this percent were rounded off as an annual turnover of 25 percent, it would mean that one person in four should have some training each year, or that refresher courses could be given profitably every four years for an entire group of people concerned with insect control in a state, county, or city health department, or mosquito abatement district.

In order to provide more effective training assistance to the states, seven field training stations have been established across the United States. These are located at Amherst, Massachusetts; Mt. Vernon, New York; Pittsburgh, Pennsylvania; Atlanta, Georgia; Oklahoma City, Oklahoma; Denver, Colorado; and Seattle, Washington. Short courses can be held at these training stations or personnel from the stations can work with individuals interested in developing courses for presentation in nearby states. These decentralized courses cut total costs of travel and per diem allowances and permit many individuals to attend who would otherwise be unable to do so.

Decentralized courses worked out cooperatively with health departments, state colleges and universities, and mosquito control organizations are typified by the four schools held in February and March 1956 in Rhode Island, Massachusetts, Utah, and Oregon. These reached a group of 90 key people engaged in mosquito control. Another quite different series of fly control schools, currently being held in Texas during July and August, 1956, will reach an estimated 250 public health biologists, sanitarians, and sanitary engineers.

During the past five years, increased emphasis has been placed on helping states develop their own training programs. In a number of cases state and local health department personnel who attended the Communicable Disease Center courses have received assistance in conducting their own short courses so that a larger number of public health workers could benefit from this type of training.

Another important question to be answered is this: "What has the Communicable Disease Center developed in the way of *new* training materials that would be of value to others?" These may be divided into two main categories: (1) audio-visual materials such as motion pictures, filmstrips and slide series, and (2) printed materials such as manuals, leaflets, training guides, and pictorial keys.

### MOTION PICTURES

Since 1942 our organization has produced approximately 100 motion pictures, about 25 of which deal with some phase of insect biology or control. A few of the outstanding motion pictures produced by the Communicable Disease Center are as follows: Space Spraying, Community Fly Control, Rat Ectoparasite Control, Organized Mosquito Control.

### FILMSTRIPS

Similarly, the Communicable Disease Center has been actively engaged in the production of filmstrips, most of which have an accompanying recorded narration. Some of the types of filmstrips are as follows: Biology of Domestic Flies, Flies of Public Health Importance, Identification of U.S. Genera of Adult Ticks, Mosquito Problems in Irrigated Areas.

The motion pictures and the filmstrips are available free of charge, on short-term loan by writing to the Film Librarian, Communicable Disease Center, 50 Seventh Street, N.E., Atlanta 23, Georgia. Each year these motion pictures reach larger audiences. An estimated 400,000 persons saw them in the fiscal year 1948, 5,200,000 in the fiscal year 1956, and a total of some 26 million viewers in the nine-year period. They have been used widely by other governmental agencies, such as the Armed Forces, the Fish and Wildlife Service, state, county or city health departments, and colleges and universities.

### PICTORIAL KEYS

The pupils in our classes vary widely in educational background and experience. Some have only a high school education, while others may have a Ph.D. in entomology. Since control programs are usually based on reducing the populations of important vectors, it has become important for control personnel to be able to identify a particular insect and



to practice species sanitation. In order to simplify the identification work as much as possible, we have worked out a series of pictorial keys which aid in the identification of common and important species of mosquitoes, flies, cockroaches, fleas, ticks, and lice. Each key is dichotomous, with the insect or the diagnostic characters arranged on the chart in key form. We have been far more interested in teaching the identification of common and important vectors with public health significance, such as the oriental rat flea, the house fly or the malaria mosquito, rather than identification of rare species which are of great interest mainly to the taxonomist and museum specialist.

The pictorial keys have been widely accepted by health departments and teachers throughout the country. Reasonable numbers of these keys have been furnished free of charge to health departments, governmental agencies, and colleges and universities for classes in medical entomology or parasitology.

### MANUALS

Over a period of years our Center has produced a number of important publications concerned with the control of insect-borne diseases. These have included such important training manuals as: *Malaria Control on Impounded Water*, *Rat-borne Disease Prevention and Control*, *Clinical Memoranda on Economic Poisons*, *Operational Memoranda on Economic Poisons*, *Pesticide Recommendations* (revised each year);

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# The Place of Contracting Organizations and Professional Supervision in the Application of Pest Control Methods

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## INTRODUCTION

It is a matter of common observation that insect control on farms is done both by the farmer and by large-scale specialist contractors. The farmer sometimes find it advantageous to do this work himself; at other times it is more advantageous to call in a contractor. The purpose of this paper is to consider the relation between the farmer's role in pest control and the contractor's role; both have their part to play. It is a matter of history that the commercial contractor has strongly influenced the farmer's approach to his own pest control operations, the capital he invests in it, the importance with which he regards it, and the way he fits it into farm routine. I propose to consider the factors which favour the expansion of commercial application and make it useful to farmers to-day, and the extent to which this special service might be improved.

At the outset it may be noted that it is impossible for our purpose to segregate insect control from other crop-spraying operations for the eradication of weeds and prevention of plant diseases, and what follows applies to the commercial practice of all forms of crop protection involving the use of chemicals.

I propose to discuss the matter under several heads. The first is the greater technical knowledge or "know-how" of the contractor, particularly with regard to new techniques. The second is his greater command of costly capital equipment. The third is the use of labour as between farmer and contractor. The fourth is the problems created by regulations for the use of toxic substances. I shall then consider how these factors have created a balance between what I may describe as the "do it yourself" practice and the "sub-contract the tricky work" method. Finally I shall consider various ways in which better use can be made of outside consultants in applied entomology and biology, both by contractors and organized agriculturalists and by both in combination.

### THE CONTRACTOR'S SUPERIOR "KNOW-HOW"

When a new method of insect control or any other crop protection practice is first proved practicable, it is of course in advance of other existing, related techniques known to farmers. Inevitably, some years and sometimes many years must elapse before it is recognized as the best practice available, becomes standard practice, and is used in such a way that good results are obtained regularly. There are always difficulties in introducing a new method. Even independent advisory services and efficient salesmanship encounter difficulties in putting complicated new techniques across to farmers—who, after all, have a whole range of problems constantly on their minds and are versed in one standard method of dealing with a problem which a new discovery may revolutionize. New knowledge—the theory of new practice—is sometimes more easily communicated than practical skill in operating the new equipment necessary to apply that knowledge in farming practice, as well as its economic management so that it achieves the promised reduction in costs. Many an inherently sound technique has suffered because it was put into operation by unskilled hands and acquired a bad reputation. So long as such a difference persists between the know-how of the contractor and the farmer, commercial application by well-trained operators and good technical management is obviously the best way of employing the new method according to the specifications laid down by the applied biologist, and of demonstrating it to the farming community. The commercial sprayer thus acts as an educational force in converting the farming community to the new method, by showing its technical feasibility and its economic advantages. The farmer and his assistants who look on and talk to the contractor's operators pick up the know-how. This includes management and operation of the sprayer, selection of the spray chemicals, prevention of wind drift, and the taking of precautions against toxic hazards to the operator and to domestic animals and game. If the commercial operator employs the services of entomologists, whose advice may have an

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important bearing on the economics of the treatment, his margin of specialized knowledge over the farmer is still greater, and, of course, this cannot be passed on by demonstration; only the desirability of taking professional advice can be.

#### THE CONTRACTOR'S SUPERIOR EQUIPMENT

Contractors in the civil engineering and building industry base their economics upon the use of much more elaborate, expensive, and specialized equipment than the small jobbing or local builder; the same large difference in output exists between the spraying and other equipment of the aerial spraying contractors and of individual farmers. The contractor's equipment would be quite uneconomic unless it could be kept for a considerable part of the year upon large-scale projects—which in the case of the building contractor means large buildings, civil engineering works, or runs of hundreds of houses, and in the case of the spraying contractor means the covering of large acreages. Between the contractor's large-scale equipment and the small hand or tractor-driven equipment, which is all that most farmers find economic, there is a category of equipment which could be owned by both; and whether it pays the grower to acquire this depends upon his capital and scale of operations. Crop spraying operations which are not necessary every year, or only for a short period in every year, will not sustain the overhead charges of keeping such equipment idle for long periods. A study of the economics of farmers' own spraying equipment by the economic section of the School of Agriculture in Cambridge showed that, in the United Kingdom on a purely cost basis, 30 acres of spraying a year would justify the purchase of a low volume sprayer by a farmer and 66 acres that of a high volume sprayer (Sturrock).

Equipment, of course, does not mean only machinery at the disposal of the contractor, but all his other facilities—water supply, safety equipment for the use of toxic chemicals, specialized services, and auxiliary equipment to ensure the accuracy of the application work.

Progressive commercial applicators try to keep their equipment more advanced—capable of modern treatments more cheaply—than that which it is at any given time economic for farmers to buy for themselves. The most progressive, in fact, develop new equipment which can do better work more cheaply than the farmer can do for himself.

#### THE CONTRACTOR'S SUPERIOR LABOUR RESOURCES

The contractor should have at any given stage in the development of pesticidal techniques an advantage in his labour resources. Good farm organization makes steady and continuous use of a permanent labour force, and does not require the employment of temporary labour for skilled operations (as opposed to seasonal demand for pickers, for example). Pest control operations, even when they can be planned, often create peaks in the demand for labour which cannot be accommodated in normal agricultural routines. In addition many treatments involve a degree of skill which is not necessary in farm workers and which, if special training were resorted to, would increase the cost of normal labour.

Where pest outbreaks occur suddenly and irregularly, the switching of the farmer's labour force to meet them may disorganize routine and delay other operations. Thus standing by for emergencies, such as fire fighting, is an important function of the contractor's labour force. His is, so to speak, a mobile reserve of farm workers; and it is his job so to arrange his business that he, too, will have no sudden peak demands which overstrain his permanent skilled and expensive labour supply—by "matching" one season or one crop or one county's need with another.

#### STATUTORY REQUIREMENTS

Regulations governing industrial pest control lay down rules for the employment of licensed and qualified operators; this also applies in many countries to aerial operations; but the licensing of spraying contractors for normal agricultural spraying and other treatments has so far not been considered necessary in the countries of the British Commonwealth. The contractor has an advantage in understanding government requirements, regulations and advisory services, which determine the country's crop protection policy.

#### USING THE CONTRACTOR

Where under one or more of these four heads the contractor is in a position to give the best (and in the early development of a new treatment the only) service, farmers have invariably called in the contractor. But this does not automatically provide the contractor

with a profitable living. The success of ventures in contract spraying and aerial application has been largely determined by the degree to which the contractor can keep his equipment and personnel utilized. This in turn has always depended on the number of insect pest outbreaks, weed infestations, and plant diseases for which a commercial service could be offered, and the density of the work in geography and time.

Chemical control of insect pests and weeds was popularized more generally among British farmers in World War II and the years afterwards because a large contract spraying organization existed which carried out commercial spraying on about a quarter of a million acres from 13 branch offices. In the counties where this service existed the sales of chemicals were many times those in counties where no such services existed to show farmers what could be done and how well it paid. As the basic technique became accepted, many farmers acquired their own equipment and sprayed according to recommendations of governments advisory service and "according to the label on the can"; nonetheless, though this contract spraying reached a peak during the early postwar period, it has only been partly superseded by farmers' own spraying because of the special advantages of contractors in some techniques as described above.

The present position is that in Britain in 1955, in order to produce our annual gross farm product of £1,285,700,000, a total of 4,395,000 acres had received at least one application of crop protection chemicals, which is 16% of all agricultural land including rough grazing. Forty-two percent of the farmers who had their land sprayed carried out their own spraying; but rather more (53%) had all spraying done by contractors, while a further 4% did some of the spraying by themselves and had some done by contractors. In terms of acres, 70% of the land sprayed was done by farmers with their own equipment, and 22% by spray contractors.

There are good reasons why contract spraying flourishes to this extent in the United Kingdom. Its agriculture—prosperous, progressive, and capable of expansion—has to contend with great seasonal variations in the appearance of the varying noxious organisms so that spraying requirements vary greatly in amount and in timing from year to year. Although the majority of farm holdings are small—80% are under 100 acres—the fields are relatively large, or at least large enough for power spraying equipment to be effectively deployed in them, in great contrast to European countries in which, under peasant ownership, the fragmentation of land has increased under the system of equal inheritance to such a degree the strip farming has made crop protection by contractors with large-scale equipment very difficult.

In the U.S.A. to produce an annual gross farm product of \$40 billion (£14,000,000,000) 57,000,000 acres were treated in 1954 with agricultural chemicals and 30% of this acreage was done by commercial application. In Canada the annual agricultural production is approximately \$4 billion (£1,400,000,000) to achieve this, 12.3 million acres were treated with chemicals and contractors were responsible for 750,000 acres or 6% of the treated acreage (Brown, 1956). In the U.S.A. and Canada aerial spraying forms a much larger percentage of the commercial application than in the United Kingdom.

The process of popularizing spraying by contractors may be seen in an entirely different environment in the Sudan. The cotton acreage which was sprayed by contract grew rapidly in the immediate post war years after experiments had established its great economic advantages and cotton growers found it easy to avail themselves of the commercial spraying service. As the techniques became better understood, many Sudanese cotton growers acquired their own sprayers and chemicals and integrated spraying with other agricultural operations. But commercial spraying did not disappear. Its share in the total volume of spraying has settled down in the proportion of three-fifths of the area sprayed as against two-fifths sprayed by the farmers themselves; all the spraying with insecticides of high mammalian toxicity remained delegated to the contractors.

### PROFESSIONAL SUPERVISION

When I discussed the advantages conferred on the contractor by his greater know-how, I mentioned his utilization of the services of the professionally qualified applied biologist. Professional scientific advice is needed in the diagnosis of many pest problems, because an accurate forecast to determine if the outbreak of a pest would be of sufficient economic importance to justify control operations is obviously of the highest value to the contractor,



just as are indications of the right treatment. Where a biological assessment is necessary before treatment or professional supervision of the treatment is desirable, the applied biologist has a contribution to make in all but the simpler cases where the agriculturalist has adequate knowledge for recognition and epidemiology of the noxious organism. This development has in the U.S.A. been much stimulated by Dr. Roy F. Smith who termed it "supervised control". The following advantages are claimed: (1) elimination of unnecessary treatment, (2) protection and utilization of parasites and predators to their fullest extent, (3) assurance that the fields are being watched by a trained entomologist and that no sudden outbreaks will destroy a crop representing a considerable investment (4) knowledge that the supervising entomologist is working for the grower and that the best and most recent entomological advice is readily available (5) each control recommendation is tailor made for each individual field.

Supervisory control, as distinct from the advisory services supplied by such government organisations as the National Agricultural Advisory Service in the U.K., has been furnished in three ways: (1) A commercial entomologist may be employed collectively by farmers through their organizations; sometimes it has been done on the lines of supervised control as suggested by Roy F. Smith—this is practiced extensively in California and the American cotton belt, and by many of the larger plantation companies in the tropics. (2) A professional entomologist may be provided as part of the service of a plant hire and distribution service offered by a chemical formulator who supplies spray chemicals, hires staff to apply them and gives an extensive advisory service. (3) Professional entomologists may advise as part of the sales service of chemical manufacturers—a variant of item (2). (4) Finally, the advice of professional entomologists is an integral part, as I have said, of a commercial application service, with or without the supply of the necessary spray chemicals.

In the United Kingdom one organization operates on the pattern of item (2). Some chemical manufacturers provide a technical advisory service. Finally, a good entomological service is available as a part of one commercial application organization.

The future of co-operation between agriculturalists and entomologists, achieved by one or other of these means, should not be in doubt. It is now widely conceded that supervision of treatments by entomologists saves many unnecessary sprayings. Entomologists however can do more than this. One school of thought believes that prophylactic spraying is liable to lower the "biotic resistance" of the ecosystem of crops, which in the long run involves the farmer in ever more heavy outlay on ever less effective treatments: entomological advice can help to ward off this damage by timing the chemical treatment so as to minimise such effects, and by applying non-selective insecticides only when the entomologist finds that the pest population has exceeded certain critical densities.

If the practical consequences of this were fully accepted, and if the integration of biological and chemical control is further developed, much more supervision of pest control methods by entomologists would be necessary and rewarding.

But before this comes about, research must be done to establish for important pests the correlation between the population density at the critical time and the yield; in addition the inter-relationship of biological control factors and the development of pest outbreaks must be quantitatively understood.

A credible beginning has been made on the exploration of the correlation between population densities and yield (Strickland, 1954), who together with many collaborators in the National Agricultural Advisory Service has measured in many fields in various parts of the country the population densities of important pests and then obtained yield data. These data were then correlated so as to obtain yield regressions from which the yield reduction for certain population densities at the critical stage could be extrapolated.

My 17 years of association with Britain's largest commercial applicators have taught me the great need to establish critical densities of insect pests and weeds in order to decide when control operations become economic. As applied entomology in Europe and North America has now reached a stage where most of our pests are understood and the pests are economically controlled, there would seem to be a fruitful field of further research in exploration of the population density and yield correlation, which would convey economic benefits to the agriculture concerned.

The professional entomologist who is called upon to make a biological assessment in order to decide what control operations should be carried out and when, is handicapped by

the lack of method and equipment by which the necessary quantitative population assessment can be carried out quickly. There can be no doubt that this lack of technical equipment for quantitative diagnosis is a stumbling block to the more general acceptance of "supervised control".

At the outset I mentioned that the control of insect applied biology should take cognizance of the inter-relationship of pests, weeds, and plant diseases of crops; from this combined study there should emerge a type of applied biologist who is able to diagnose and advise on the treatment and prognosticate the development of the outbreak of *all* the important noxious organisms which beset the crops with which he is concerned. It is obvious that this would result not only in considerable economies of labour and transport, but it would also lead to much more integrated recommendations leading to the avoidance of one-sided treatments that cause unfavourable repercussions on the noxious organisms, currently often dealt with by yet another chemical treatment.

The need is for a crop protection expert who on a sound agricultural training has a good knowledge of the important pests, weeds, and plant diseases of the crops with which he deals, knows their method of chemical control and has mastered the methods of quantitative assessments of the noxious organisms concerned. The Germans call such a man a *Planzenarzt*, the verbatim translation of which would be Crop Physician; at the Third International Crop Protection Congress in Paris the name "phytiate" was proposed, but no good English term has so far been accepted. There are few academic institutions which have a sufficiently wide syllabus to train such a specialist, although this type of applied biologist would meet a demand which is likely to increase in future. It might be argued that this form of academic training involves too extensive a syllabus, but investigation shows that it would not be more extensive than that demanded by a degree in medicine. It seems urgent to provide for this type of training because the increasing complexity of agricultural science enhances the growers' desire to delegate the supervision of the more complicated pest control operations and the bioassay prior to such control operations to a qualified applied biologist.

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### DISCUSSION

F. H. JACOB. Supported contention that prognosis was a vitally important part of the advisers' duty. It was, however, fraught with technical difficulty. The determination of the losses resulting from pest attack deserved careful attention, for entomological literature is rich in loss data that is little more than guesswork—often not particularly inspired.

Real losses could often be surprising and recent work (organised by A. H. Strickland) on one important pest, the fruit fly (*Oscinella frit* L.) had indicated an annual hidden loss of some £11.5 million in England and Wales.

Regarding Dr. Ripper's suggestion that the best kind of adviser would be a specialist in several disciplines, Mr. Jacob said that in England and Wales the advisory Entomologists were also responsible for nematology.



# The Development of Commercial Entomology in the United States

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## ABSTRACT

*The term "Commercial Entomology" is here used to designate the activities of graduate entomologists in the employ of profit-making organizations. This is in contrast to "Official Entomology" which includes the employment of entomologists by federal, state, county, and other non-profit agencies or institutions.*

*If "Official Entomology" is considered to have started with the appointment of Townend Glover as Entomologist of the U.S. Patent Office in 1854, it took just 50 years for the first graduate entomologist to become associated with a profit-making organization.*

*The growth in numbers of entomologists employed by business and industry is indicated and some of the reasons for the increase are analyzed. The continually widening field of activities is traced and the more important kinds of business which employ graduate entomologists are briefly described.*

*Although it is estimated that only about 15 percent of all professional entomologists are presently connected with profit-making organizations, an increasing number of trained entomologists will undoubtedly enter the commercial field as time goes on. The rate of increase will depend to a considerable extent on the imagination and aggressiveness of entomologists themselves, in pointing out how business can profit by a greater use of their specialized knowledge and services. The development of more adequate curricula in our colleges and universities can contribute greatly to better enable students to enter the commercial field and attention should be given toward this end.*

*The term "Commercial Entomology" is here used to designate the activities of graduate entomologists in the employ of a profit-making enterprise. In contrast, entomologists who are employed by official non-profit government, state or county agencies and by privately endowed institutions may be said to be engaged in "Official Entomology".*

*If official entomology is considered to have started with the appointment of Townend Glover in 1854 as Entomologist of the newly-created Bureau of Agriculture of the U.S. Patent Office, together with Asa Fitch as State Entomologist of New York in the same year, it required just 50 years before a graduate entomologist became employed by a profit-making organization. This pioneer is Dr. Otto H. Swezey who, in 1904, left Ohio State University to become an Assistant Entomologist in the Experiment Station of the Hawaiian Sugar Planters Association. After about 30 years of active service, he continued as a consulting entomologist for nearly 20 years longer and as this is written is living in retirement in California at the age of 87.*

*Prior to 1920, we have record of only 14 entomologists entering the commercial field. From 1920 to date, it has proved impossible to obtain statistics on growth to any great extent. However, the following, based on over 250 names of individuals for each of whom the starting date is known, will give some idea of the rate of increase of entomologists entering business organizations:*

*During 1920-29, over 40 started in and although some went with growers' organizations and into structural pest control work (PCO's), most joined the growing pesticide industry.*

*The decade of 1930-39 added only some 30 odd more, but the actual number was undoubtedly larger. The range of activities also was expanded.*

*The decade of 1940-49 considerably more than doubled the number starting during the previous 10 years and the lines of work became even more diversified.*

*During the first 6 years of the 1950's, nearly 10 percent more started in commercial work than during the whole of the previous decade and a number started during the first half of 1956.*

The most generally accepted figure is that there are now some 4500 individuals who follow the profession of entomology. As to how many of these are in commercial work of some kind, it has proved difficult to determine with any real accuracy. The best estimate that can be made is that there may be 650—700. If the higher figure is accepted, it would appear that commercial entomologists constitute around 15 percent of the total number of entomologists.

### FIELDS OF COMMERCIAL ACTIVITIES

Starting about 50 years ago, entomologists have entered an increasing number of different kinds of businesses, some of which have only rather recently found use for specialized entomological training. Although these several fields are not always subject to clear definition and may be somewhat overlapping, the principal ones may be designated as follows:

Pesticide Industry; Structural Pest Control Operators (PCO's); Growers Organizations; Supervised Pest Control; Commercial Crop Dusting and Spraying; Forest Products Industry; Grain and Milling Trade; Seed Growers; Canning Companies; Tree Experts; Nurserymen; Dairy Industry; Biological Supply Houses; Commercial Parasite Production; Railroads; Consulting Entomologists.

There follows a brief discussion of these varied activities.

### THE PESTICIDE INDUSTRY

Since early in its history, the pesticide industry has employed entomologists to test new compounds in the laboratory and field, and to improve and expand the uses of the older ones. As time went on, entomologists were also employed as technical salesmen and more latterly as sales managers and in other administrative positions.

In what might be called the "pioneer period"—the years prior to 1920—five of a total of 14 entomologists who entered commercial work joined pesticide manufacturers. They were: Paul Jones, with Balfour-Guthrie in 1912; S. W. Foster, with General Chemical Company in 1913; V. I. Safro, with the then Kentucky Tobacco Products in 1914; J. E. Graf, with the same firm in 1916; and A. J. Gunderson, with Sherwin-Williams in 1919.

In the decade of 1920–29 at least three times as many entomologists entered the commercial field as during all previous years, and about two-thirds of these joined pesticide firms. In 1930–39, at least three-quarters of all entomologists entering the commercial field went into the pesticide industry, and during 1940–49 about 80 to 85 percent. This percentage has dropped to about 60 since 1950, due to a larger number having entered other phases of commercial enterprise. Although it has proved rather difficult to state with considerable accuracy, it is probable that as high as seventy percent of all commercial entomologists are presently employed by the pesticide industry.

Without doubt, one of the most important factors in increasing the use of graduate entomologists in this industry was the establishment, in 1920, of the Crop Protection Institute by W. C. O'Kane on invitation of the National Research Council. This organization created an opportunity for chemical manufacturers to screen out and develop new compounds for their possible pesticidal value by placing fellowships with leading Universities. In each case, a graduate entomologist was selected to conduct the investigations which led to his Ph.D. under the direction of competent members of the faculty.

This program has proved notably successful in developing useful pesticides and in firmly establishing a number of firms in the pesticide manufacturing business. So successful, indeed, has the program of the Institute been that according to R. J. Norton, its Director, "It may be conservatively stated that seventy-five per cent of the current agricultural chemical industry had its inception in and has been nurtured to successful establishment by the Crop Protection Institute". A total of upwards of twenty-five entomologists have held CPI fellowships through to completion of the Ph.D. and most of these have come to hold key positions in industry and some in the land-grant colleges.

A survey of its member firms, very recently made by the National Agricultural Chemicals Association, indicates considerably increased employment of entomologists in the pesticide industry during the next few years. This expectation is due to the inevitable expansion of this industry. The need for food by a rapidly expanding population—not only in this country but throughout the world—will, in itself alone, necessitate the use of greater quantities of pesticides. Furthermore, the increasing diversity of pesticides and



complexity of control measures will make necessary more entomologists in research, product development, technical sales and administrative positions. Almost every firm that manufactures, formulates, and sells insecticides now has one to many entomologists on its staff. Included in this category are a number of the farmers' cooperatives, oil companies, and fertilizer manufacturers.

#### STRUCTURAL PEST CONTROL OPERATORS (PCO's)

Nearly 100 years ago, Solomon Rose started a business in Cincinnati for the control of insects which infest houses and other buildings. During the course of many years, similar firms started up throughout the country and they came to be known as "exterminators", a designation still in fairly common use. Although for a long time their methods were necessarily somewhat crude and largely unscientific, results were apparently effective enough to gradually increase public acceptance.

In 1933, a group of operators, who contract to control insect pests in and around buildings, organized an Association which in 1936 took its present name, National Pest Control Association. In 1933, the membership was 158; at present it is over 900 and some large firms have branches in most of the leading cities and in many smaller towns.

E. R. Barber was apparently the first graduate entomologist to go into this business, when he started the Barber Laboratories in New Orleans in 1922. It seems that no other entomologists entered this field until George Sanders and V. B. Durling started The Sanders-Durling Entomological Service, which included termite control, in New York City in 1930. They were followed by George Hockenyos who started the Sentinel Laboratories in 1933 in Illinois. W. E. McCauley worked with him in 1934. W. K. Delaplane organized the Illini Pest Control in Illinois in 1942 and H. L. Fellton went with Orkin in 1946.

A most forward-looking move was taken by J. J. Davis in 1946, when he organized a 4-year course at Purdue, especially designated to fit entomologists to enter the Pest Control Operators business. About 20 students have graduated from the class of 1950 to date. Including these, an increasing number of other trained entomologists have been entering this industry, mostly in the last 10 years, and it is now estimated that there are at least 125 in this field. One firm reports 25-35 entomologists at present who joined from 1940 to date; another 7 who joined in 1952-54; no specific figures are available as to the others.

#### GROWERS' ORGANIZATIONS

Sugar producers were among the earliest to employ entomologists for the reason that there was little or no official help available for the solution of their insect problems. Swezey in Hawaii has already been mentioned. H. T. Osborn joined the same organization in 1913 and remained until 1924. He then became entomologist for the United Sugar Companies in Mexico, an American concern, from 1925-27 and for the Aguirre Sugar Company in Puerto Rico, 1928-31. R. H. Van Zwaluwenburg, 1921-24, was with the United Sugar Companies of Mexico. In 1910, D. L. Van Dine assisted by T. H. Jones, started entomological investigations for the newly-created Experiment Station of the Puerto Rican Sugar Producers' Association. In the same year, A. C. Maxon was made Director of the Experiment Station of the Great Western Sugar Company where he remained until 1945, having become the leading authority on insects affecting sugar beets.

C. E. Pemberton joined the Sugar Planters' Experiment Station in Hawaii in 1919 and became Emeritus Entomologist in 1953, and Van Zwaluwenburg went there in 1924 and is still on the active staff. W. D. Pierce was entomologist of the Victorias Milling Company in the Philippines in 1927-30.

An important project on sugar cane insects was started in 1924 by D. L. Van Dine as Director of an experiment station set up under the auspices of the Tropical Plant Research Foundation (USA), by the Cuba Sugar Club, an organization of Cuban sugar producers. C. F. Stahl joined at the same time and was succeeded in 1929 by U. C. Loftin, at which time L. D. Christensen also joined the staff. H. K. Plank had come down in 1926. Due to ruinous sugar prices, the station was closed in 1932 just about the time the mosaic problem was almost solved.

In the citrus industry, Sunkist Growers has had an outstanding group of entomologists who have directed and improved control measures on their extensive citrus plantings in

California and Arizona. The Bureau of Pest Control was started in 1920 with the employment of R. S. Woglum who retired late in 1948. J. R. LaFollette was added to the staff in 1924 and retired toward the end of 1955. W. E. Landon joined in 1926 and retired the end of 1949. H. C. Lewis joined in 1929 and succeeded LaFollette as Chief Entomologist, in which capacity he still acts. Two other entomologists on the staff are W. E. Shilling and C. P. Teague, the latter of whom joined in 1949.

Pure Gold (originally the Mutual Orange Distributors) has H. L. Thomason in charge of its Field Department who, though not a graduate entomologist, is college trained and advises growers on pest control as well as cultural practices.

It is quite possible that other smaller citrus growing companies have, or have had, entomologists, but their names have not been determined. In Mexico, D. L. Crawford was Horticultural Expert for the Gulf Coast Citrus Association, 1912-14.

R. N. Chapman, an entomologist, became Director of the Pineapple Producers Association in Hawaii in 1930 and in the same year Walter Carter became Head, Department of Entomology of the Pineapple Research Institute. D. L. Crawford was Technical Consultant to the Pineapple Canneries in Mexico, 1945-48.

The California Fig Institute has as Director of Research, R. M. Warner who started in 1946 and an assistant in entomology, D. F. Pratt, who started in 1954.

The California Walnut Growers Association does not have a specially trained entomologist on its staff, but O. L. Braucher has been handling entomological work along with other production problems in the Field Department. The Saticoy Walnut Growers Association of California had S. E. Flanders as entomologist, 1923-29.

The United Fruit Company has long had a program of research in tropical agriculture which has naturally included insects which have affected their extensive plantings. Among the entomologists employed have been the following: Marston Bates and A. J. Johnson, 1928-31; J. M. Deal 1934-36; Colin Campbell 1952-55; F. S. Roberts since 1949. Several other entomologists, acting as consultants on special problems in the recent past, have included C. E. Palm, J. L. Brann, and J. E. Dewey of Cornell and R. G. Oakley of USDA 1950-51.

From 1923-30, A. W. Morrill was agricultural economist and entomologist of Mexican West Coast Vegetable Association, a large American organization, where he successfully carried out large scale control operations on tomatoes by the use of airplanes.

For lack of a better place, the tobacco industry may be placed in this category. Only one firm—the American Tobacco Company—employs an entomologist, Paul Vinzant, who started his connection in 1940. Control procedures are devised and carried out to reduce to a minimum insect populations in the cigar and cigarette factories, as well as in the stored tobacco, and control procedures are prescribed for pests of cigar wrapper tobacco grown on company properties in Connecticut and Cuba. Factors which may affect flavor by field applications of insecticides to tobacco are studied in cooperation with state and federal workers.

#### SUPERVISED PEST CONTROL SERVICES

In recent years there has come into operation, largely in California, a type of commercial service to large growers which is known as Supervised Pest Control. Several large farmers and individual organizations in California now employ one entomologist, and two or three employ from one to seven during the summer season to advise them on pest control procedures on such crops as alfalfa hay, alfalfa seed, and cotton. There is usually a flat charge of a dollar or two per acre. One such service started in 1952 and it is reported that at least three or more started this year. An idea of the extent of some of these operations is indicated by the fact that one service now handles 5000 acres, another 7500 last year, and another 25,000 to 35,000 acres.

In 1945, Joe Schuh started a service with Kenneth Gray in Oregon for berry, pea, and potato growers. Since 1948, Schuh has been working in another area mostly for potato and clover growers.

Extensive operations of what amounts to the same kind of service have developed in several of the cotton states. Here the service is usually referred to as "Cotton Scouting" or "Cotton Insect Scouting".

It appears that this kind of commercial service may have reached its most extensive development in Arkansas and a wide-spread program is by now in operation in which the College of Agriculture recruits the scouts, regulates their activities, and gives them periods of training. K. P. Ewing was apparently the first cotton scout in Arkansas where he scouted for insect infestations on a large plantation in the season of 1920. The present program in cooperation with the college, however, started in 1926 with J. G. Horsfall (now in Connecticut) as the first scout. Others started scouting in 1932, 1933, 1939, and 1949. There were 8 scouts in 1950, 20 in 1951, a steady growth to 50 in 1955 and a jump to 80 plus in 1956, during which season they will scout about 140,000 acres or 8-10% of the cotton acreage of Arkansas. Some are graduate entomologists but most are students, although the scout is fully qualified to recommend control measures.

The University of Missouri is reported to be starting a plan similar to that in Arkansas, by placing 3-5 students in the field this summer. The Delta and Pine Land Company of Scott, Mississippi, has employed Morris Blocker since 1952. At least six other experienced cotton entomologists, including E. W. Dunnam, scout extensive cotton acreages in Mississippi and there is an undetermined amount of scouting in Texas and some in certain other states, including Louisiana. This type of service is certainly susceptible to very considerable growth and it may be expected to increase considerably in the next few years.

#### COMMERCIAL CROP DUSTING AND SPRAYING

In the 1920's, certain individuals and small companies began to contract with some of the larger farmers to apply pesticides by ground equipment or by airplane. A service charge was made for an agreed amount per acre per application, the farmer supplying the pesticides. In some cases the applicator furnished whatever pesticides the farmer wanted applied. For some time past, most of the applications have been made by airplane. Commercial applicators have steadily increased until now it is estimated there are more than 400 throughout the United States.

Such extensive applications of pesticides would seem to call for the employment of entomologists in various technical capacities, but it appears that very few indeed are directly connected with these operations. This is probably due to the establishment, especially during the past several years, of special seminars held by a number of the state agricultural colleges for personnel of crop dusting and spraying companies. At these sessions the latest methods of crop pest control, as well as the factors of safety of use of pesticides to applicators, crops and livestock, and the public in general, are discussed by the entomologists of the college staff.

The largest firm in this type of business, Delta Air Lines, organized its service in 1924 and soon began to hire graduate entomologists increasing to a considerable number. Mr. Woolman, Extension Entomologist for Louisiana, was the first Chief Entomologist and later became President of the parent company. B. R. Coad joined the firm in 1931 in charge of the so-called "Dusting Division" which was later expanded into the Agricultural Division of which he is still the manager. He reports the Division has a number of trained fieldmen, one of whom joined in 1926, but it appears that only a few of them are actually graduate entomologists.

#### THE FOREST PRODUCTS INDUSTRY

Companies which grow trees for lumber and the manufacture of other forest products have for many years employed graduate foresters, many or most of whom have taken one or more formal courses in forest entomology. Apparently, it is only recently, however, that any of these organizations have started to employ forest entomologists as such. Although there may be others, to date we have record only of the following firms with starting dates of their entomologists:

Bowater Paper Company (Hiawasse Land Company)—R. C. Mason, February 1956; Gaylord Container Corporation—R. E. Heinz, January 1953, H. Galusha, February 1955, G. Hatchell, February 1956; Union Bag and Paper Corporation—R. E. Lee, early 1955; Weyerhaeuser Timber Company—V. F. McCowan, June 1952, J. Rudinsky, June 1953—June 1955, N. Johnson, summer of 1955 and from June 1956. Kimberly-Clark Corporation reports forest entomologists have been in their employ continuously since 1951, but presently there is only J. B. Cody, who started in June 1955.

A most informative paper was presented by R. E. Lee of Union Bag before the joint meeting of the Entomological Society of America and the Association of Southern Agricultural Workers at Atlanta, Georgia, February 6–8, 1956 entitled "The Duties of a Company Forest Entomologist". In this, he predicts that "in the future, wood-using companies will give even greater attention to their insect losses and more and more of them will employ forest entomologists".

#### THE GRAIN AND MILLING TRADE

For some years past, milling and grain processing companies have employed college graduates with more or less training in entomology for the control of such limiting factors as moisture, fungi, rats, and stored grain insects. These technologists are variously referred to by titles such as: mill biologists, mill sanitarians, and economic biologists, but some have the title of entomologist. It is probable that most of these spend their major effort on insect control. One company reports 8 graduate entomologists and there may be a total of 25 or 30 in this industry. The "Sanitation Coordinator", who is a graduate entomologist, of one of the largest milling companies reports that their tendency is definitely to hire more trained entomologists as openings occur.

#### SEED GROWERS

It appears that seed companies have found but little use so far for graduate entomologists. This is probably due to their having members of their firm who, though having majored in other agricultural subjects, nevertheless have had some formal training in entomology.

We have record of only the following firms employing entomologists: Associated Seed Growers, C. D. Harrington, started in 1940; and Northrup, King and Company, G. D. Moore, started in 1952. Whitman Seed Company of Yuma, Arizona, is reported to employ H. A. Hunt as a Consulting Entomologist.

#### CANNING COMPANIES

Although there are many canning companies, very few employ college graduates who have majored in entomology. In fact, we know of only two that do. This is probably due to the fact that many have technical members of their staffs who have had some undergraduate training in entomology, followed by considerable field experience in insect control. More important, however, are annual schools for the canners' fieldmen, which have long been held by many state colleges of agriculture at which the latest practices in pest control for canning crops are thoroughly discussed by competent members of the staff.

California Packing Corporation employs nine graduate entomologists, one of whom has been with the organization 18 years, 3 for 10 years, 1 for 7 years, 1 for 2 years, and 2 for 1 year each. Dr. Caldis, Director Agricultural Research, although trained in plant pathology has, as he understates it, "in the last 30 years had more than a casual acquaintance with insects and entomologists". He joined Cal-Pack in 1926. The Director of Agricultural Research of the Green Giant Company, J. G. Martland, who joined the company in 1941, is an entomologist.

#### TREE EXPERTS AND ARBORISTS

Although there are several hundred firms which care for shade trees and ornamental plantings, nearly all are too small and localized to have found need of graduate entomologists.

The Secretary-Treasurer of the National Shade Tree Conference writes: "I believe that in the future there may be more tree concerns employing graduate entomologists and plant pathologists, but I do not look for a very rapid development along this line. In most states, arborists can get entomological information rather readily from their experiment stations or universities, both in the form of research and extension material".

The F. A. Bartlett Tree Expert Company had, as Entomologist and Director of the Bartlett Tree Research Laboratories, E. P. Felt from 1928 until the time of his death in 1943. S. W. Bromley was Assistant Entomologist, beginning in 1929, and later Entomologist until his death in 1954. The research Laboratories now employ two entomologists—E. J. Duda, who started in 1951, and D. F. Bray, starting in 1955. The Davey Tree Expert



Company has, as Technical Advisor, F. R. Lancaster, a plant pathologist with a minor in entomology, and W. A. Jeffers who joined as an entomologist in 1953.

#### NURSERYMEN

But little information has been available as to employment of graduate entomologists by nursery firms. In the opinion of one large nurseryman, there may not be more than a dozen or so firms that employ full-time graduate entomologists or plant pathologists to head up their pest control work.

The Armstrong Nurseries of Ontario, California, had, as Entomologist in charge of Pest Control, Leo R. Gardner from 1922-26 and have had one pest control man in their employ ever since. Presently Dave Almquist is in charge of pest control, starting in 1952. Preceding him was Harold Jeancon from 1945 to 52, and prior to him for several years Clifford Pappe. The President of these nurseries states that this type of work is essential in his operation and that they could not do without it. In view of this, it would seem that more of the larger nurserymen, at least, could use to advantage such technically trained men.

It may be of interest to record that the writer was employed as an entomologist by the Chase Bros. Nurseries of Rochester, New York, during the three summers of 1913-15, in connection with the injury to peach nursery stock by the tarnished plant bug.

#### THE DAIRY INDUSTRY

In 1945, E. M. Searls became entomologist for the National Dairy Products Company for which organization he supervises the control of insects affecting dairy cattle and those that contaminate plants in which dairy products are processed.

#### BIOLOGICAL SUPPLY HOUSES

Ward's Natural Science Establishment has employed one or more entomologists over a number of years. Dr. Elliot A. Maynard was Head of Entomology from 1925-1929. Dr. A. B. Klotz was Head of the Department of Entomology from 1931-34 and Dr. Glenn A. Richards ran the Department for a time on an unofficial basis. R. L. Post was Assistant Entomologist, 1932-34, and Head of Entomology, 1934-39.

#### COMMERCIAL PARASITE PRODUCTION

A unique business enterprise, based on highly specialized techniques in handling insects, was established in California in 1930 by A. W. Morrill. This he called the California Insectaries, Inc. A considerable business was developed in the mass breeding and wide-spread sales of parasites and predators of several major pests of sugar cane and several other crops. This continued until his death in 1954.

#### RAILROADS

For many years, railroads have had in their employ trained agriculturalists to assist in increasing agricultural products for shipment over their respective lines. Undoubtedly, many of these specialists have had some formal training in entomology and some may have been graduate entomologists. The only ones, however, in this latter class, of whom we have definite knowledge, are H. H. Stage, who was Entomologist for the Cottonbelt Railway from 1920-30, and A. W. Morrill, who was Entomologist for the Southern Pacific Railway of Mexico from 1921-23. William F. Turner, a graduate entomologist, was "Horticultural Agent" of the Central of Georgia Railway 1923-29.

#### CONSULTING ENTOMOLOGISTS

A rather small number of entomologists are presently listed as Consultants and certain others have been so listed in the past, but there never have been more than a few, all told.

There have not been the opportunities for consultants in entomology that there have been in such fields as engineering and chemistry, since free advice on pest control to farmers—the principal potential clients—has always been available from federal, state, and county agencies. As insecticides came into wider use and spray schedules became more complicated, official help was supplemented by the technical representatives of pesticide manufacturers.



With the increasing number of pesticides and the complexity of problems concerning their use, it may well be that greater opportunities will present themselves for properly trained and experienced consultants with growers' organizations and other businesses and organizations which have insect problems, as well as with the pesticide manufacturers themselves.

### CONCLUSION

Today, as for some time past, little or no difference in professional standing is recognized between entomologists who are employed by industry and those who hold positions in official agencies and organizations. It is therefore difficult for a great many entomologists, especially the somewhat younger ones, to realize that this has not always been so. Those few who prior to 1920 left official positions to enter industry, and even the larger number who did so during the 1920's, were considered by many of the profession, especially the older members, to have lowered their professional standing and their opinions were considered likely to be less unprejudiced than before. This was particularly so when they became involved in direct sales promotion.

This writer still recalls that when he left an Assistant Professorship at Cornell in 1921, 35 years ago, to improve product performance, field operations, and sales literature for a well-established manufacturer of insecticides and fungicides, he was made to feel by a number of his colleagues in official positions that his status was not quite as respectable as before.

As has been previously indicated, in the case of several of the businesses that employ entomologists, the prospect is that they will find use for an increasing number of entomologists in the future. This is likely to be true, to a greater or lesser extent, in all of the categories of business referred to in this paper. It is to be expected also that trained entomologists will invade fields which are not as yet using their special knowledge.

Although various kinds of business activities are becoming increasingly aware of the value of entomological knowledge, the increase in the use of entomologists will undoubtedly depend to a considerable extent on the imagination, vision, and aggressiveness of entomologists themselves. Insects, directly or indirectly, affect a great many of the products and services we utilize, as well as our health and comfort, and we have only really just begun to explore how we can much more effectively counteract their effect on these things. This presents an opportunity—as well as an obligation—to the teachers of entomology to so modify and expand their curricula as to better fit entomologists to become of greater value to business in the broad sense.

It might be suggested, however, that one contributing approach would be for one of our largest Departments of Entomology to experiment with a series of seminars, open to senior and graduate students. These sessions would be conducted largely by department managers of large pesticide firms to discuss the objectives and the workings of such matters as research, product development, market analysis, production, distribution, and sales.

This paper represents a first attempt to review the development of the entry of graduate entomologists into commercial enterprises and their present status in such activities. It is realized that this summary is incomplete, especially in certain of the categories of employment. The writer will welcome corrections, additional information, and suggestions.

# Recent Advances in Plant Protection Methods Under Tropical Conditions in Uttar Pradesh, India<sup>1</sup>

By A. S. SRIVASTAVA  
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Uttar Pradesh was the first among the states of India to organize a Plant Protection Scheme in 1947 with a view (a) to organize control operations against the major pests and plant diseases on a field scale in different parts of the state (b) to help the farmers in undertaking such control measures themselves and to assist them in obtaining insecticides, fungicides, spraying and dusting equipment promptly and cheaply (c) to give them technical advice with practical demonstrations (d) to warn the farmers against the possibility of pest and disease outbreaks (e) to persuade them to adopt timely measures against such outbreaks and, (f) to enforce measures for preventing the entry and spread of pests and diseases.

This service has been placed under the charge of the Entomologist to Government, U.P., who is also Officer-in-Charge of Plant Protection Service. The headquarter of this Service is situated at Kanpur. It has several Plant Protection centres located at Meerut, Agra, Bareilly, Bakshi-Ka-Talab, Lucknow, Banaras, and Gorakhpur and has 14 sub-centres at Dehradun, Bijnor, Saharanpur, Mainpuri, Haldwani, Jhansi, Aligarh, Etawah, Fyzabad, Gonda, Azamgarh, Mirzapur, Allahabad, and Shahjahanpur. All the districts are covered by one or other centre or sub-centre or the headquarters. At each centre and sub-centre, staff, pesticides, machines and other equipment are placed so that they may serve as effective bases for conducting operations, as well as for other types of Plant Protection work. The Plant Protection Centre at Bakshi-Ka-Talab is used for imparting instructions in methods of Plant Protection to officers of various extension Departments. At present the staff totals 231.

During the last eight years this Service has conducted large scale operations against a variety of pests listed below and has at the same time imparted training to thousands of Village Level and District-cum-Pooled staff. These control operations have created a consciousness in the minds of farmers, who are now themselves carrying out the control operations in their fields, if required. During the first few years the control operations against pests and diseases were conducted free of cost to farmers and orchardists, but since 1951 this Service is collecting part of the cost of control operations against certain pests and plant diseases from farmers and fruit growers. During the year 1955, control operations against twelve pests and diseases (including mangohoppers and late blight) are being charged for. The policy has been to gradually withdraw the free service in respect of pests and diseases.

Various recommendations for pest and disease control:—

Sl.	Pest or Disease	Months in which found in pest form	Treatment given
1.	<i>Pyrilla</i> ( <i>perpusilla</i> W.)	April-October	Dusting with 5% BHC dust @ 20 lbs. per acre or spraying with 0.25% BHC
2.	Termites ( <i>Odontotermes</i> sp.)	All the year round	Dusting of burrows with 5% BHC dust @ 20-25 lbs. per acre before sowing cane crop
3.	Grasshoppers ( <i>Hieroglyphus</i> sp.)	July-September	Dusting with 5% BHC or DDT @ 20-25 lbs. per acre followed by raking into the soil

<sup>1</sup> Grateful acknowledgement is made to Dr. S. B. Singh, Director of Agriculture, Uttar Pradesh, Lucknow, for his interest in this work.

Sl.	Pest or Disease	Months in which found in pest form	Treatment given
4.	'Gujhia' ( <i>Tanymecus indicus</i> F.)	July, November and December	Dusting with 5% BHC or DDT @ 20–25 lbs. per acre followed by raking into the soil
5.	Cotton leaf roller ( <i>Sylepta derogata</i> F.)	July–September	Spraying with 0.5% DDT or BHC
6.	Jute semi-looper ( <i>Anomis sabulifera</i> G.)	June–August	Dusting with 5% BHC @ 20–25 lbs. per acre
7.	Hairy caterpillars ( <i>Amascata</i> sp. and <i>Diacrisia obliqua</i> W.)	June–August	(1) Destroy the egg masses and caterpillars (2) Dusting with 10% BHC or spraying with 0.5% BHC
8.	Red pumpkin beetle ( <i>Aulacophora foveicollis</i> .)	February–September	(1) Dusting with Pyrodust @ 10–15 lbs. per acre (2) Dusting with 5% DDT @ 20 lbs. per acre (3) Dusting with sodium fluosilicate mixed with ash (1:8) @ 20–25 lbs. per acre
9.	Aphis	November–December	(1) Spraying with tobacco soap decoction (1:1:10) (2) Spraying with 2 to 3% fish oil rosin soap
10.	Potato tuber moth ( <i>Gnorimoschema</i> <i>operculella</i> L.)	—	Store potatoes in sand dusted with 10% DDT @ 2 ozs. per 100 mds. of potato
11.	Late blight of potato ( <i>Phytophthora infestans</i> )	November–March	Spraying with 0.25–0.3% Perenox solution
12.	Early blight of potato ( <i>Alternaria solani</i> )	October–March and July and August	Spraying with 0.25–0.3% Perenox solution
13.	Singhara beetle ( <i>Galerucella birmanica</i> )	June–November	Dusting with 5% BHC 20–25 lbs. per acre
14.	Gundhy bug ( <i>Leptocoriza varicornis</i> F.)	June–November	Dusting with 5% BHC 20–25 lbs. per acre
15.	Blast disease ( <i>Helminthosporium</i> sp.)	—	Spraying with 0.3% Pere- nox solution
16.	Mangohoppers ( <i>Idiocerus</i> spp.)	February–April	Spraying with 0.25% DDT
17.	Mealy bug ( <i>Drosicha mangiferae</i> )	February–April	Spraying with .15% nico- tine sulphate plus 1.25% sesame oil white plus .25% soap
18.	Lemon butterfly ( <i>Papilio demoleus</i> L.)	Throughout the year	Dusting with 5% BHC

Sl.	Pest or Disease	Months in which found in pest form	Treatment given
19.	Citrus psylla ( <i>Diaphorina citri</i> )	—	Spraying with tobacco soap decoction
20.	Citrus canker ( <i>Xanthomonas citri</i> )	Throughout the year	Spraying with 0.3% Perenox solution
21.	Citrus-wither-tip	Throughout the year	Spraying with 0.3% Perenox solution
22.	Bark-eating caterpillar ( <i>Inderbela</i> sp.)	Throughout the year	Injecting kerosene oil into borer holes and then plastering the holes with mud
23.	Woolly apple aphid ( <i>Eriosoma lanigerum</i> Hausm.)	August, October, November January and February	Spraying with 2 to 3% fish oil-rosin soap
24.	Seed borne diseases	—	Seed dressing with Agrosan GN (1:500 on wheat and 1:320 on other cereals)
25.	Foot rot of pan ( <i>Phytophthora parasitica</i> )	—	Spraying the crop and soil with 0.3% Perenox solution





# The Contribution of Biological Studies to the Control of Anthomyiid Flies of Economic Importance

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## ABSTRACT

Closely-related Anthomyiid flies with a common host plant show important differences in their biologies and habits. *Hylemyia brassicae* Bouché emerges from the over-wintering diapause in April and is injurious mainly in spring and summer, while *H. floralis* Fall. emerges after mid-summer and attacks brassicas in late summer and autumn. *H. antiqua* Mg. infests onions mainly in June and July but attacks by *H. cilicrura* Rond. often occur about a month earlier. *H. brunnescens* Zett. which attacks leaves and shoots of *Dianthus*, is univoltine. The larvae feed in late autumn and winter, and pupation takes place in March and April. The flies emerge in May and June but do not lay eggs until September and October.

These observations on the annual cycles of the well-known Anthomyiid flies demonstrate that biological and ecological studies have an important contribution to make to pest control. The success of protective measures may be largely determined by the time of application, and correct timing and the form of the insecticide to be used may depend on detailed biological knowledge of the species, especially in its field aspects.

The Anthomyiid root-flies and leaf-miners are well-known throughout the north temperate zone as major pests of vegetable crops. Their individual life-histories have been carefully observed and the nature of the field attacks has been accurately described, but there is less information on the times of attack by the various species and their relative importance at different seasons.

For some time Canadian entomologists have known that there is a mixed population of primary pests among the root-maggots on onions and brassicas, and that the first necessity for field workers is to be able to identify all the developmental stages of the common injurious species. Brooks (1951) of the Canada Entomological Service at Saskatoon has made a notable contribution to this aspect of the field problem and his work on the identification of the root-fly larvae is well-known. My work has been complementary to his, and has been concerned with the biological differences between the species, as a further aid to their recognition in the field.

## BIOLOGICAL DIFFERENCES BETWEEN THE CABBAGE ROOT FLY (*Hylemyia brassicae*) AND THE ONION FLY (*H. antiqua*)

The biological differences are generally best studied in the field, but this is not always possible. When I wished to study onion fly in the field I found that although it was thought to be common and generally distributed in England, its distribution was in fact very erratic and the presence of onion crops in no way ensured that onion flies were part of the fauna of the district. This has been observed also in Michigan (Merrill, 1951).

When multivoltine Anthomyiid flies are bred in a laboratory one is impressed by the common features in their life-histories and habits and it can readily be understood how it came to be stated in the literature from both sides of the Atlantic that onion flies and cabbage root flies have a similar cycle. By studying the two species comparatively I have found that their morphological differences are paralleled by biological differences.

Both species are capable of breeding continuously under suitable temperature conditions, but in the north temperate zone the falling temperature in late summer and autumn induces a pupal diapause. The most outstanding biological difference between the two species lies in the times at which the flies emerge from the puparia after the winter diapause.

My records over several years showed that when the two species were wintered together in an insectary the cabbage root flies emerged mainly in April and the onion flies mainly in May, both species having an emergence period of approximately one month. The times of emergence varied slightly from year to year, but a difference of about a month between their respective peak periods of emergence was constant in the south-east of England. After puparia of both species had been exposed to frost in an outdoor insectary

they were transferred to a laboratory with a temperature of 10–20°C. Both species emerged before their normal time, but again the cabbage root flies began to emerge about a month earlier than the onion flies and there was no overlapping of the two emergence periods. These observations indicate that the two species have different temperature requirements.

Another biological difference between the two species supports this view. In both, the ova are undeveloped when the flies emerge from the puparia and the time required for the maturation of the eggs depends on nutrition and temperature. When both species were kept at 25°C and fed on a solution of milk, sugar and water, the pre-oviposition period for cabbage root flies was 5–6 days and that for onion flies was 10–16 days.

Taken together, the differences in the time of emergence after the winter diapause and in the length of the pre-oviposition period at a constant temperature, suggested that the two species were adapted to living in different climates, the cabbage root fly attaining full reproductive maturity under cooler conditions than those necessary for the onion fly. Field observations supported this. I have found for several years that cabbage root flies begin oviposition on their host plants during the last week of April, and reliable observers in England record that onion flies begin to lay eggs about the end of May.

#### THE ROOT-MAGGOT COMPLEX ON ONIONS

Once it was established that attacks by onion fly were not likely to be seen in the field before the second half of June, it was necessary to account for the injury by Anthomyiid larvae that had often been seen in May. From the literature on onion fly and a knowledge of the life-histories of the Anthomyiid root-flies it can be said with reasonable certainty that these early attacks are made by seed-corn maggots (*H. cilicrura*). I have not been able to induce seed-corn maggot attack on onions in the field but I have observed a severe infestation of shallots in May, and early serious attacks by seed-corn maggots on onions have been observed in Michigan (Merrill and Hutson 1953).

The confusion of seed-corn maggots with onion maggots is understandable. The two Anthomyiid flies are closely related. Their eggs are identical in appearance but slightly different in size, and their larvae differ mainly in the number of processes of the anterior spiracles. The two species are distinct biologically. The seed-corn flies emerge in April and begin egg-laying in the latter part of the month, while onion flies are not active until a month later. The habits of the two species also differ. The seed-corn flies are attracted by the condition of the soil and lay their eggs in it irrespective of the crop; onion flies appear to lay their eggs only on *Allium* crops.

These differences in the life-histories and habits of the two species should be taken into account when control measures are devised, a suggestion that has already been made by workers in Michigan. Under English conditions an insecticidal seed-dressing may protect the germinating seeds and young seedlings of onions from the wandering maggots of the seed-corn fly, but it seems doubtful whether a seed-dressing will still be effective a month later when onion flies are active, particularly as onion maggots may reach their feeding sites without coming in contact with the insecticide on the seed-coats or in the soil surrounding them. Certainly, in experiments with insecticides for the protection of onions against attacks by root-maggots it seems important to know what species are present on the crop, and to assess separately how the several species are affected by the treatments.

#### THE ROOT-MAGGOT COMPLEX ON BRASSICAS

Brassicas are also attacked by two closely-related species: cabbage root flies (*H. brassicae*) and turnip root flies (*H. floralis*). The eggs of the two species are similar in appearance but differ slightly in size, and the larvae have many morphological characters in common though they can usually be distinguished in the field with a hand lens. Flies and larvae of the two species have similar egg-laying and feeding habits but they are distinct biologically.

Both species have the capacity for continuous breeding under suitable temperatures, but under natural conditions both pass the winter in a pupal diapause. With these species also, the most important biological difference lies in the time of emergence after the winter diapause. In England cabbage root flies begin to emerge in April; turnip root flies emerge from the end of June to early September. This biological difference seems to be as constant as the morphological differences. The hibernating pupae of cabbage root flies are sensitive to small increases in the temperature of their environment and the emergence of the flies

takes place while the soil temperature is still low. Turnip root flies do not emerge until after midsummer, and a prolonged exposure to a constant temperature of 25°C, beginning some time before it occurred naturally, made no observable difference in the time of their emergence.

This difference in the time of emergence from over-wintering puparia results in a corresponding difference in the time of larval attacks. My observations on the annual pattern of oviposition by the successive generations of cabbage root flies have shown that attacks are most intense in spring. The number of eggs laid varies from year to year with the weather conditions, but the annual pattern stays fairly constant. After about the first week of August there are relatively few eggs of cabbage root flies about their host plants. The observations on egg-populations have been supported by observations on the larval populations. These also show that the highest infestations occur in spring, and that relatively low populations are found on the plants at other seasons.

I have not been able to observe attack by turnip root fly in the field, but observations in Scotland have shown that very high larval populations of turnip root fly occur in August and September, and this is what one would expect with a species whose overwintering generation emerges so late in the season. Owing to cropping practice, the same plants are not usually exposed to the main attacks of both species. Turnip root fly attack usually occurs on turnips, but in captivity the larvae feed on other brassicas. Extensive decay in turnips in autumn is sometimes associated with cabbage root fly attacks but I have never found large numbers of maggots present at that time. When a severe attack by turnip root fly occurs, 60-70 larvae per turnip may be found.

The biological data on turnip root fly should be of value in attempts to define its geographical distribution in Eurasia and North America and should also assist field workers to distinguish the two species in the field.

#### THE CARNATION FLY AND THE SPINACH STEM FLY

Another pattern of Anthomyiid life-history occurs in the carnation fly (*H. brunescens*) which is well-known in Europe as a serious pest of carnations. Previous workers state that they have not observed the complete annual cycle, but they have assumed that the flies have two or three generations a year. In consequence of this erroneous assumption, it has not been possible to anticipate the attacks and recommend appropriate treatment.

Carnation flies emerge in early summer from about the middle of May to the middle of June. They differ from the other species I have described in that they require about three months to reach reproductive maturity, that is, from June to September. The long period between emergence and oviposition is not one of aestivation. The flies are active and they feed, but they seem to spend most of the time at rest in the shade. Oviposition takes place in September and October. The eggs are laid singly on the leaves and in the leaf axils of cultivated species of *Dianthus*. After an incubation period of one to two weeks, the eggs hatch and the larvae feed as leaf-miners or they tunnel into the shoots. The length of the feeding period varies. Some larvae finish feeding by about the end of November and enter the soil for hibernation. Others remain exposed in the leaves and shoots throughout the winter. Immature larvae feed in winter when the temperature is suitable. Most of the larvae have finished feeding by the middle of February. Larvae that complete their development in the autumn hibernate in the larval stage. Pupation takes place from mid-March to mid-April and the pupal stage lasts about two months.

The carnation fly is therefore univoltine. It has an active adult life throughout the hottest period of the year, that is from May to October, and oviposition does not take place until autumn. The larval life continues from September to April, and feeding larvae are able to survive exposure to freezing. Now that the life-cycle of the carnation fly is understood, it should be relatively simple to secure the protection of valuable plants from attack.

The carnation fly also has a closely-related species in the spinach stem fly (*H. echinata* Ségué) with many similar morphological characters but a markedly different cycle of generations. The two species have similar oviposition habits and lay eggs with the chorion similarly sculptured. Larvae of the spinach stem fly are also leaf-miners and stem-borers. The spinach stem fly, however, passes the winter in the pupal stage and emerges in the latter part of April. Oviposition takes place in late April and May and the larvae are fully

fed by early June. First-generation flies emerge in June and early July and there are one or more later generations.

The relationship between the carnation fly and the spinach stem fly is as close as that between the other pairs of species that I have discussed, but yet they have different host plants. Apparently this is not always the case. Hering (1951) states that the spinach stem fly normally feeds on Caryophyllaceae, but my work has been mainly among vegetable crops and I have found the species only on spinach. It has been suggested that there is a close relationship between the plant families Chenopodiaceae and Caryophyllaceae, and this would support the observation that they are alternate hosts for some phytophagous species.

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# Biological Control of the Japanese Beetle Especially with Entomogenous Diseases

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## ABSTRACT

One of the most successful attempts to control an important insect pest by biological means was made with the Japanese beetle, *Popillia japonica* Newm., through the colonizing of entomogenous bacteria and nematodes, and imported parasitic insects. These bacteria are now widely distributed in the eastern United States and probably play the most important role in maintaining the beetle population at a relatively low level. The imported parasitic insects probably rank next in maintaining the biological balance. The entomogenous nematodes are most restricted in distribution. Lesser but important contributions are made by other entomogenous bacteria, fungi, protozoans, rickettsias, and other parasitic insects. In fact, all organisms that attack the Japanese beetle are a part of the biological complex that is functioning to limit the growth of potentially destructive populations of the beetle. With the many ecological variations throughout the area infested by the beetle, it is important to have several types of organisms participating in maintaining the biological balance.

Biological control of the Japanese beetle is a relatively inexpensive method of reducing the population over a large area. Since it is a natural method of control, the organisms attacking the beetle tend to establish themselves permanently under favourable conditions, to increase in effectiveness, and to spread of their own accord into surrounding areas. They are essentially harmless to man, warm-blooded animals, and plants. Finally, the bacteria, nematodes, and parasitic insects used to control the Japanese beetle are not antagonistic one to the other, but work in a complementary manner to establish the balance of nature in the favor of man. The success of this long-range program is indicative of what can be accomplished through careful, thorough fundamental research and the close cooperation of Federal and State entomological agencies in colonizing the organisms over a wide area.

When the Japanese beetle *Popillia japonica* (Newm.) was carried accidentally, probably in nursery stock, from its native Japan to southern New Jersey some time prior to 1916, it found a generally favorable climate and other conditions suitable for its development. It found over 200 kinds of plants to satisfy its voracious appetite, including orchard fruits, small fruits, field and garden crops, and ornamental trees, shrubs, and herbaceous flowers; it discovered large areas of permanent turf for oviposition and development of the grubs, and there were practically no known important enemies. From an area of less than one square mile in 1916 (Smith & Hadley 1926) the beetle spread steadily in all directions until by 1956 it had invaded about 100,000 square miles, extending from Maine to South Carolina and Westward to Ohio. It had also been carried in commerce and become established in several isolated areas beyond this region of natural distribution.

The history of the Japanese beetle in the United States is in contrast to its record in Japan. The beetle is not an economic pest in its native land because it has never been sufficiently abundant there. This may be due in part to the limited number of host plants and breeding areas, and the many natural enemies that keep it under control (Hadley & Hawley 1934). During the first 12 years following its discovery in the United States, the annual broods of the beetle increased in magnitude and in importance. In southern New Jersey the population became so dense that there were annual flights across the Delaware River into the downtown shopping and market districts of Philadelphia, Pa. Millions of beetles flew out to sea and annoyed fishing parties even 5 miles off shore, and later were washed up dead and dying, in windrows on the beaches of the shore resorts. Shade trees were shorn of their splendor, orchard trees had clusters of beetles devouring ripening fruit, grapevines were skeletonized, and garden plants were stripped of their foliage and blooms. In some places the turf had a population of 150 grubs per square foot. Insecticides of that day were only partially effective in protecting crops and ornamental plantings from the devastating hordes. No practical method was available for preventing severe damage by the grubs to valuable turf; grub-proofing by the use of residual insecticides was developed later. With the beetle a scourge in the land and with research to develop control measures being con-



ducted at a frantic pace, the size of the annual broods began to decline, until by 1945 the pest was of only minor economic importance except in a few isolated areas. This cycle of the rise and decline has been repeated with modifications as the beetle invaded new areas.

The introduction of the Japanese beetle into the eastern United States upset the biological balance of nature. The insect-feeding birds, small terrestrial mammals, and the few predaceous and parasitic insects indigenous to the region were not sufficient to cope with the 20- to 30-fold reproductive potential of the beetle. Birds feeding on the adult beetles are the domestic chickens, ducks, turkeys, and guineas, and a few wild birds, among which are the purple grackle (*Quiscalus quiscul* L.), the European starling (*Sturnus vulgaris* L.), the cardinal (*Richmondia cardinalis* L.), the meadow lark (*Sturnella magna* L.), the catbird (*Dumetella carolinensis* L.), the house sparrow (*Passer domesticus* L.), the robin (*Turdus migratorius* L.), and the ring-necked pheasant (*Phasianus colchicus* L.). Birds also dig up and devour large numbers of grubs, especially when fields are being cultivated and when the grubs are close to the surface in grasslands. The domestic fowl, the European starling, the purple grackle, the crow (*Corvus Brachyrhynchos* Brehm.), and the gulls (*Larus* spp.) are the most important grub-devouring birds (Cory & Langford 1944, 1955; Hadley & Hawley 1934; Polivka 1950; Smith & Hadley 1926). The terrestrial mammals feeding on grubs are the common mole (*Scalopus aquaticus* L.), the star-nosed mole (*Condylura cristata* L.), the short-tailed shrew (*Blarina brevicauda* Say), the skunk (*Mephitis mephitis* Schreber), and possibly the pine mouse (*Pitymys pinetorum scalosoides* Audubon and Bachman) (Sim 1934). Of the native predaceous insects, the Carabidae, Tabanidae, and Therevidae (Hallock 1929) will attack the grubs when they encounter them, and the Asilidae (Bromley 1945, Hallock 1929) and Formicidae (White 1940) have been observed feeding on both adults and grubs. Practically none of the native parasitic insects attack the beetles. A wasp, *Tiphia intermedia* Mall. (Hadley 1938), parasitizes sporadically a small proportion of the grubs. In recent years a fly, *Ptilodexia* sp., has been observed attacking them. Although these native enemies of the Japanese beetle have from time to time reduced the population within a limited area, their efforts are too sporadic and too restricted to have much effect on the population in a region.

When the beetle began to decline in southern New Jersey, it was thought that the reduction might be due to the deficiency of rainfall when the eggs were hatching in the soil. Since the small grubs cannot endure prolonged desiccation (Ludwig 1936), the amount of rainfall during the summer is an important factor in the year-to-year fluctuations in the population (Fox 1939, Hawley 1949). However, the decline continued even when the rainfall was favorable for the development of the grubs. Climatological data showed that dry and wet summers occurred with about the same frequency during the rise of the beetle as during its decline. It was evident that some other factor was restoring the biological balance in the area. Two new biotic agents—imported parasitic insects and native diseases—were becoming established, and it is known now that they were largely responsible for the progressive decimation of the beetle population.

### IMPORTED PARASITIC INSECTS

Although imported parasitic insects are a part of the Biological complex controlling the Japanese beetle, only brief reference will be made here to their importation, establishment, and role in this complex in the United States. From 1920 to 1933 a diligent search was made in the far East for insects that were parasitic on the Japanese beetle and related Scarabaeidae. During this period 1-1/2 million insects of 34 species, including Larvaevoridae (*Tachinidae*), Pyrgotidae, and Scoliidae, were collected and shipped to the United States (Clausen *et al.* 1927, 1933; Gardner & Parker 1940). Much fundamental research was conducted on the Biology of these species and to determine their adaptability to their new environment (Balock 1934; Brunson 1934; Burrell 1931; Holloway 1931; King 1925, 1931, 1937, 1939; King *et al.* 1927; King & Holloway 1930, 1930a; King & Parker 1950). Five of them—*Dexilla ventralis* (Ald.), *Prosenia siberita* (F.), *Hyperctena aldrichi* Mesnil (*Centeter cinerea* Ald.), *Tiphia popilliavora* Rohw., and *Tiphia vernalis* Rohw.—have now become established in the eastern United States and have become a permanent part of the Fauna of the region. The most effective and widely distributed of these species is *T. vernalis*, which has *Popillia quadriguttata* as its normal host in Korea and *P. chinensis* in China (Gardner & Parker 1940). *T. vernalis* attacks overwintering grubs of the Japanese beetle in the spring, and *T. popilliavora* grubs of the new brood in late summer.

The female *Tiphia* wasp searches out a grub in the soil, stings it to cause temporary paralysis, and attaches a single egg to its under side. The maggot-like larva hatching from the egg remains attached to the grub, sucking blood from it. The young parasite attains full growth within a few days, by which time the grub has been consumed, except for the head capsule. The parasite larva then spins a strong, watertight, silken cocoon within the earthen cell formerly occupied by the grub and the following year emerges as an adult (King & Parker 1950).

Beginning in 1921 and continuing for 25 years, *T. vernalis* was colonized in cooperation with the State entomological agencies in Connecticut, Delaware, Maryland, Massachusetts, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, Vermont, Virginia, and West Virginia, and in the District of Columbia. *T. popillivora* was released in eight of these States. Recent surveys have shown that these parasites are widely established throughout this area; in many localities they are so abundant as to be noticed readily by the layman. Under favorable conditions *T. vernalis* has parasitized over 60 percent of the Japanese beetle grubs in an area (King & Parker 1950); *T. popillivora* is somewhat less effective. There is no doubt that these two wasps have important parts in the biological complex controlling the Japanese beetle. The other imported parasitic insects have a lesser role.

### ENTOMOGENOUS DISEASES

Diseases were found occasionally among Japanese beetle grubs as early as 1921 (Hawley & White 1935, Smith & Hadley 1926). Although these diseases were not identified specifically, they were determined to be mainly of bacterial and fungous origin. By 1933 large numbers of diseased grubs were found in certain areas in New Jersey, Delaware, and Pennsylvania (Hawley & White 1935); in June of that year 12 percent of the grubs found in field surveys were diseased (Hadley & Hawley 1934). The numerous maladies were classified tentatively as the "black group," the "white group," and the "fungous group" (Hadley & Hawley 1934). The early work on diseases of the Japanese beetle, however, was limited in scope and not conclusive; intensive studies were not undertaken until 1930.

### PROTOZOANS

Five protozoan genera have been found in the hindgut of the Japanese beetle grub—*Polymastrix*, which is primarily an insect parasite, and *Retortemonas*, *Monocercomonas*, *Monocercomonoides*, and *Tetratrichomastix*, which have been found in association with many invertebrate and vertebrate hosts. The association of these protozoans with the grub seems to be a mutualistic one, causing the host no harm, and probably benefiting it (Kowalczyk 1938).

In the course of a survey at Cape Charles, Va., in 1938, several grubs of *Strigoderma pygmaea* (F.) and *Phyllophaga gracilis* (Burm.) were found with a microsporidian infection of the fat bodies. The same microsporidian seemed to be involved in both species. Attempts to produce infection artificially in grubs of these species and in grubs of the Japanese beetle were not successful (Dutky & White 1940). In 1950 Japanese beetle grubs infected with a microsporidian were found at Warrenton, Va., but since then no cases of this disease have been found there, or elsewhere within the area infested by the Japanese beetle. The possibilities of a microsporidian disease for the control of the beetle have not been investigated.

### NEMATODES

In the spring of 1929 Japanese beetle grubs infected with a nematode, *Neoaplectana glaseri* Steiner, were found at Haddonfield, N. J. Later that year parasitized pupae and adult beetles were found in that area (Glaser & Fox 1930). Probably the nematode had some other insect as its normal host and had adapted itself to the Japanese beetle (Steiner 1929). Although a search was made in other parts of New Jersey and in Pennsylvania, it was not found at that time in any other area.

*Neoaplectana glaseri* is a general parasite of insects, but it is not pathogenic to plants. It parasitizes grubs of *Anomala orientalis* Wth. at least as much as those of *P. japonica*. Other susceptible insects include several species of *Phyllophaga*, *Autoserica castanea* Arrow, *Macroductylus subspinosus* (F.), *Cyclocephala borealis* Arrow, *Cotinis nitida* (L.), *Xyloryctes satyrus* (F.), *Cotalpa* sp., *Graphognathus* spp., and *Pyrausta nubilalis* (Hbn.) (Glaser et al. 1940). Attempts to infect armyworms, silkworms, and house fly larvae were

not successful, indicating a degree of specificity for the nematode (Glaser 1931). *N. glaseri* also attacks adult Japanese beetles in the soil (Glaser *et al.* 1940).

The infective second-stage nemas enter the grub by way of the mouth, develop two or three generations within the body, and destroy the grub by feeding on the tissues. They continue to develop in the cadaver of the grub until most of the tissues have been consumed. The grub at death is flaccid, of a reddish-brown color, and swarming with nemas, most of them in the second stage ready to invade the soil and infect another host. As many as 2,400 infective-stage nemas have been recovered from a single grub, but the usual number is about 1,500 (Glaser 1931, 1932). Since this nematode continues to develop in the dead host, it has been suggested that *N. glaseri* is a species in transition from a free-living saprozoic mode of existence to a parasitic one (Glaser *et al.* 1940).

It was demonstrated that *N. glaseri* can be established in an area where it does not occur naturally, and when so established can cause a high mortality of the grubs (Glaser 1932). In field experiments 0.3 to 82 percent of the grubs were parasitized, depending upon the conditions (Girth *et al.* 1940). The optimum conditions are (a) a minimum soil temperature of 15.5° C. (60° F.) at a depth of 1-½ inches, (b) a soil moisture of 20 percent or higher without flooding, (c) turf or other permanent cover, and (d) a dense host population. The nematode has survived for 24 years in the field when the host population was maintained by stocking with grubs periodically, and it was able to maintain itself for 14 years with a low host population. It is capable of sustained existence for at least 1-½ years in the absence of host insects (Glaser *et al.* 1940).

The nematode is spread slowly through the soil by its own movement and by nematode-infected grubs. Other soil-inhabiting organisms, such as other insects and earthworms, probably assist in its dispersion. It may be carried considerable distances by parasitized adult beetles (Girth *et al.* 1940, Glaser & Farrell 1935), by birds and small mammals, and in the movement of soil by wind, water, or man.

With the development of methods for propagating *N. glaseri* in large quantities on artificial media (Glaser 1931a, McCoy & Glaser 1936, McCoy & Girth 1938), in 1940 a program was undertaken to establish colonies at 3½-mile intervals over New Jersey (Girth *et al.* 1940). This colonization program was completed in 6 years. A similar colonization program was carried out at 100 sites in Maryland (Cory & Langford 1955). Sufficient time has not elapsed for a complete appraisal of the effectiveness of these programs, but there are indications that the nematode is an important factor in the decline of the beetle. This is one of the few instances in which nematodes have been colonized for the control of an insect pest. *N. glaseri* is not effective under so wide a range of conditions as the bacterial diseases and the parasitic insects, but when introduced into habitats suitable for its development it is a worthwhile parasite of the Japanese beetle.

*Neoaplectana chresima* Steiner has also been found in grubs of the Japanese beetle. The biology of this species is similar to that of *N. glaseri* (Glaser *et al.* 1942). Other nemas infecting grubs of this beetle have been found occasionally in the field. Several species of bacteria have been found associated with a number of types of nematodes; some of these bacteria have been isolated but their identity is still uncertain. The possibilities of *N. chresima* and the other nematodes for control of the beetle have not been investigated.

#### FUNGI

*Metarrhizium anisopliae* Metsch., the green muscardine, was found attacking Japanese beetle grubs in 1921 (Smith & Hadley 1926). Since then grubs infected with this fungus have been found from time to time throughout the beetle-infested area. Although *M. anisopliae* is widely distributed naturally, the incidence of this disease is usually very low (Hawley & White 1935). The fungus grows readily on artificial media. Some of these cultures remained viable for 6 months but lost their potency in 12 months. Inoculations of small field plots produced infected grubs. It appears, however, that this fungus is so dependent upon the proper temperature and moisture that artificial dispersion is impractical, except where these optimum conditions prevail. In such places the fungus apparently exercises its full effectiveness without the help of man. There is no doubt that the sporadic and small but continuous occurrences of this disease among the grubs have a beneficial effect even though they may rarely bring the beetle under control.



*Metarrhizium glutinosum* Pope was found also to be pathogenic to the grubs of the Japanese beetle. This fungus probably has about the same limitations as *M. anisopliae*.

*Isaria densa* Auct., a pathogen of *Melolontha* spp. in France, infected grubs introduced into inoculated soil under experimental conditions, but the disease has not been used successfully in the field (Hawley & White 1935).

*Beauveria bassiana* (Bals.) Vuill. was found to be pathogenic to the European corn borer (*Pyrausta nubilalis* Hbn.) under certain conditions (Bartlett & Lefebvre 1934, Bradley 1952). A method of propagating this fungus in large quantities was developed for use in control of the Japanese beetle (McCoy & Carver 1941). The spores appear to have little pathogenicity to the grubs, but adult beetles allowed to feed on foliage sprayed with a dilute suspension of them soon became infected. Healthy beetles kept in close association with infected beetles contracted the disease (Rex 1940). Although this disease occurs sporadically in areas where the fungus was colonized, it is probably of little importance in the control of the beetle.

### BACTERIA

In 1921 it was recognized that bacterial diseases existed among Japanese beetle grubs (Smith & Hadley 1926). In 1926 and 1927 several species of bacteria were isolated from affected grubs, some of which proved to be highly pathogenic. Only a few bacteria have been studied extensively, the most important being the organisms causing the milky disease of the grubs.

Milky disease was discovered in New Jersey in 1933, when a few abnormally white grubs were found in the field, and microscopic examination showed that the blood of these grubs was teeming with bacterial spores (Hawley 1952). By 1938 milky disease was prevalent in the older areas infested by the beetle, but it was not found in the more recently infested areas (Hadley 1938). In 1940 it was demonstrated that this disease was caused by two species of bacteria, designated temporarily as type-A and type-B milky disease organisms (White & Dutky 1940), and later described and named *Bacillus popilliae* Dutky and *B. lentimorbus* Dutky, respectively (Dutky 1940). *B. popilliae* is the better known and probably the more important.

The origin of the milky disease organisms is not known. It is probable that they are obligate parasites of the grubs of the Scarabaeidae, those of the Japanese beetle being their most common host (Beard 1945, Fox 1937, Wheeler & Adams 1945).

*Bacillus popilliae* does not affect insects other than Scarabaeidae, or earthworms, warm-blooded animals, or plants (Hadley 1948, 1948a). The knowledge of the susceptibility of other Scarabaeidae is based principally on tests with a limited number of individuals obtained incidentally during field surveys for Japanese beetle grubs. Grubs of the following species have been found infected naturally by this organism (Adams 1949, Dutky 1941, Tashiro & White 1954, White 1947): *Amphimallon majalis* Raz., *Anomala orientalis* Waterh., *Autoserica castanea* Arrow, *Cyclocephala borealis* Arrow, *Phyllophaga anxia* (Lec.), *Ph. fraterna* Harris, *Ph. fusca* (Froel.), *Ph. futilis* (Lec.), *Ph. hirticula* (Knoch), *Ph. inversa* (Horn), *Popillia japonica* Newm., *Strigoderma pygmaea* (F.). In the laboratory the following additional species became infected after a suspension of the spores had been injected into the blood: *Anomala innuba* (F.), *A. lucicola* (F.), *A. oblivia* Horn, *Aphonus castaneus* (Melsh.), *Dichelonyx* sp., *Diplotaxis* sp., *Macroductylus subspinosus* (F.), *Pelidnota punctata* (L.), *Phyllophaga bipartita* (Horn), *Ph. congrua* (Lec.), *Ph. crassissima* (Blanch), *Ph. crenulata* (Froel.), *Ph. drakei* (Kby.), *Ph. ephilida* (Say), *Ph. forbesi* Glassgow, *Ph. forsteri* (Burm.), *Ph. glaberrima* (Blanch), *Ph. gracilis* (Burm.), *Ph. horni* (Sm.), *Ph. implicita* (Horn), *Ph. micans* (Knoch), *Ph. minuta* (Burm.), *Ph. quercus* (Knoch), *Ph. rugosa* (Melsh.), *Ph. tristis* (F.), *Phytalus georgianus* Horn, *Strigoderma arboricola* (F.), *Trichiotinus* sp. No infection developed after injection in *Lichnanthe vulpina* (Hentz), *Cotinis nitida* (L.), or *Cyclocephala immaculata* (Oliv.). Thus, 40 species of white grubs were found to be susceptible and 3 species appeared to be resistant. These findings indicate the possibility of utilizing this bacterium for the control of white grubs other than those of the Japanese beetle. In areas where native white grubs are abundant, it might be colonized before they are invaded by the Japanese beetle.

The pathogenicity of the milky disease organisms to the grubs of the European chafer (*Amphimallon majalis* Raz.) was demonstrated in laboratory and field tests in 1946. Several strains of the organism have become established in the chafer-infested area in western

New York, and it appears possible that eventually the chafer will be controlled through the use of disease (Tashiro & White 1954).

The several strains of *Bacillus popilliae* seem to differ in their virulence to the various Scarabaeidae. *P. hirticula* and *P. rugosa* did not become infected in soil inoculated with spores grown in the blood of *P. japonica*, but developed infection in soil containing spores from a field-infected *P. hirticula* grub. *Cyclocephala immaculata* did not become infected when injected with spores from *P. japonica*, but grubs of *C. immaculata* with a bacterium similar to *B. popilliae* were found in the field (White 1947). The New Zealand strain of the organism infecting grubs of *Odontria zealandica* White was found to have a low pathogenicity to grubs of *Amphimallon majalis*. The spores produced in the blood of *P. japonica* had about the same virulence to grubs of that species and *A. majalis*, but after successive passages through *Amphimallon* they became more potent against *Amphimallon* and practically lost their potency against *Popillia*. This may be the method by which the various strains of *B. popilliae* have developed in nature.

The spore stage of *B. popilliae* normally occurs in the soil. As the Japanese beetle grubs work their way through soil, feeding on roots and other vegetable matter, they ingest the spores along with other material (Hadley 1948, 1948a). The spores germinate, penetrate the wall of the gut, probably through the Malpighian tubules, and enter the blood as vegetative rods (Beard 1945). Infection does not begin until the vegetative rods appear in the blood. A grub may become infected also by biting a diseased grub and obtaining a mouthful of infectious blood, or by hypodermic injection. The vegetative bacteria multiply rapidly in the blood, giving it a cloudy appearance. Just what induces sporulation of the bacteria is not known, but it occurs when the vegetative forms become exceedingly numerous. Sporulation continues until all these bacteria have reached the definitive spore stage, completing the cycle. At this time the blood of the grub assumes its milky-white appearance (Beard 1945, Dutky 1940). The maximum number of spores found in a grub is 20 billion (Dutky 1940), but the average is about 2 billion (Beard 1945, Cory & Langford 1955).

Grubs, pupae, and adults of the Japanese beetle are susceptible to infection. In some grubs the bacteria passed from grub to pupa, remained viable through histolysis and histogenesis, and produced an acute diseased condition in the adult (Langford *et al.* 1942). A close relationship exists between the development of the disease and the ability of the host to metamorphose. It is possible for an infected grub to molt before the disease has completed its development. If at the normal time of pupation the bacterium is not in its final stage, metamorphosis proceeds in an apparently normal manner. After the blood of a grub becomes milky it does not usually develop further (Beard 1945, Langford *et al.* 1942).

The virulence of *B. popilliae* for the Japanese beetle is far less than that of *B. larvae* Ches. and Chey. for the honey bee (*Apis mellifera* L.). Only about 25 spores of the latter bacterium are required to produce foul-brood in 50 percent of the bee larvae, whereas to cause similar infection of the grubs 11,000 spores of *B. popilliae* injected parenterally or about 2,100,000 spores per gram of dry soil by ingestion are required (Beard 1944). In contrast to many entomogenous septicemic diseases, which kill the host within a few hours or days, *B. popilliae* passes through a slow, methodical course of development, and the infected grub does not always die upon completion of the bacterial cycle. Many grubs live for weeks, or even months, after their blood has become loaded with the spores, particularly at low temperatures. It is not uncommon for grubs that become diseased late in the fall to live until they resume activity in the spring. Not only do diseased grubs remain alive, but they continue to feed. As death approaches, they become less active and then moribund. At this time the circulation of the blood stops and the bacteria settle to the bottom of the body cavity (Beard 1945).

The spores of *B. popilliae* are very resistant to adverse conditions and may remain viable in the soil for many years, ready to infect successive generations of grubs. The spores remain alive after having passed through the digestive tracts of birds and small mammals that have eaten diseased grubs (White 1940a). When spore-containing blood of a grub was dried in a film on glass slides, the spores retained their viability and potency for several years; pH less than 5 appeared to reduce their potency (Beard 1945). Spores exposed to ultraviolet light (Beard 1945) or to sunlight (White 1946) were reduced in potency. However, when the spores were mixed with talc and chalk many of them were protected from



the adverse effect of light (White 1946). There was some loss of potency when spores were heated to temperatures above 90° C. (194° F.) or kept under refrigeration in a water suspension (Beard 1945).

The temperature range of development of *B. popilliae* seems to be between 16° and 36° C. (60.8° and 96.8° F.) (Dutky 1940). Temperature has an important bearing on its effectiveness in the field. When the temperature of the soil is below 21.1° C. (70° F.), a rapid buildup of the disease is impossible. In Virginia a definite buildup occurred in one year (White & Dutky 1940), but farther north a much longer period was required—in Connecticut (Garman *et al.* 1942) and in New York (Adams & Wheeler 1946) three to four years.

The principal source of *B. popilliae* infection is probably soil contaminated by the disintegrating bodies of grubs containing mature spores. The spores thus incorporated in the soil eventually may be ingested by a grub. A dense population of grubs and a high inoculum potential favor the rapid spread of the disease (Beard 1945, White 1940a). The successful use of *B. popilliae* depends upon its transmission from grub to grub and consequent increase and spread. Since the disease may be transmitted from the grubs to the adults, the adult beetles with their migratory habits also aid in the dispersion (Langford *et al.* 1942).

The parasite *Tiphia vernalis* is a carrier of viable spores. While searching for grubs in infested soil, this wasp becomes contaminated with spores, and when it goes to another site, some of the spores are deposited there (White 1943). The disease is also spread by other insects, birds, skunks, moles, and mice (White & Dutky 1940).

Many bacterial pathogens of man, animals, and plants can be grown on artificial media and thus produced in large numbers. The cultural possibilities of *B. popilliae* have been studied in Federal, State, and private laboratories since 1939 (Hawley 1952). Several media gave consistently high yields of the vegetative forms of the bacterium, which are not suitable for distribution in the field, but until recently none of them were adequate for sporulation (Dutky 1947). In 1955, too late for the colonization program, sporulation was induced by transferring the vegetative cells grown on a complete medium to a starvation medium, or by raising the temperature after a good vegetative growth had been obtained on the complete medium (Steinkraus & Tashiro 1955).

In the absence of a suitable artificial medium in 1939, a process was devised for producing the spores in the blood of the grubs and incorporating them in a dust mixture for storage and distribution in the field. The essential features of this method have been patented (Dutky 1940a). In brief, the process is as follows: Third-instar grubs are inoculated with a known dose of spores, usually 1 million per grub, and incubated until the disease develops. The infected grubs are then ground up, the spore content is determined, and the mixture is used to impregnate chalk. This concentrate is dried and diluted further with talc. The final product is standardized to contain 100 million spores per gram of dust (Dutky 1942, Hadley 1948, 1948a, White & Dutky 1942). Over 180 thousand pounds of this spore dust have been produced at the Moorestown, N. J., laboratory.

In 1939 an extensive program of colonization of *B. popilliae* was undertaken in cooperation with various State and Federal agencies to accelerate the spread and buildup of the disease. The manner of distribution was based on the observation that under favorable conditions the organism can be established by placing 200 million spores per spot at intervals of 10 feet (White & Dutky 1942). The spore dust was usually applied with a hand corn planter of the rotary type adjusted to deliver 2 grams per spot (White & McCabe 1943). It was also mixed with fertilizer (White 1948). The manner of applying the dust and the size of the area treated varied according to the requirements of the situation and the type of program required by the different States. Reports have been made regularly by Federal workers on the progress of this program (White & Dutky 1942, White & McCabe 1943, 1946, 1951). In addition, a number of the cooperating State workers have reported upon their share in the program, among which are Connecticut (Garman *et al.* 1941, 1942, 1943, Schread 1944), Delaware (Chada *et al.* 1942, Rice 1943, Stearns *et al.* 1941), Maryland (Cory 1940, Cory & Langford 1944, 1955), Missouri (Denning & Goff 1944), New York (Buchholz 1942, 1943, Smith 1941, Smith & Daniel 1942, Wheeler 1943, 1943a, Wheeler & Adams 1945), Ohio (Polivka 1950), Pennsylvania (Light 1943), Rhode Island (Eddy 1943),

and Virginia (French 1941). The area treated increased each year until 1953, when the program was discontinued, more than 167,000 pounds having been applied at 123,000 sites. Nearly 94,000 acres had been treated in 251 counties in 14 eastern States and the District of Columbia. In addition, 15,500 pounds had been used in treating properties owned or maintained by the Federal Government (Hawley 1952). A significant but unknown number of acres had been treated by private individuals with spore dust produced under license and distributed by commercial concerns. Furthermore, the disease is extending itself naturally and giving much natural control without the aid of man.

Although widespread distribution was not begun until 1939 and only a small portion of the land in a given area had been treated, by 1945 there was a high incidence of the disease in Connecticut, New York, New Jersey, Delaware, and Maryland, where treatments had been applied early (Hawley & Dobbins 1945). There have been many favorable reports on the reduction of grubs following the application of the spore dust; only a few of them will be mentioned here. At Cape Charles, Va., in 1939 the grub population was reduced 90 percent from July to September (White & Dutky 1940). At West Chester, Pa., there was a 75-percent decline from September 1938 to June 1939, and at Springfield, N. J., an 85-percent decline from September 1939 to June 1940 (White 1941). At Perryville, Md., in surveys covering six broods from 1939 through 1949, there were reductions of 86 to 94 percent (White & McCabe 1950). In Ohio surveys in 1949 showed reductions ranging from 4 to 70 percent in different areas (Polivka 1950). At all these localities there were significant increases in the incidence of disease. By 1955 *B. popilliae* was distributed within the area generally infested by the Japanese beetle.

*Bacillus lentimorbus*, the bacterium causing type-B milky disease, often occurs in association with *B. Popilliae*. The biology of this species is similar to that of *B. popilliae* (Dutky 1940). However, it has a more limited range of temperature for its development, 16° to 30° C. (60.8° to 86° F.), and there has been considerable difficulty in using it to prepare satisfactory dust mixtures. In nature it is an important factor in the biological control of the Japanese beetle, and several strains are showing promise against *Amphimallon majalis* (Tashiro & White 1954).

A phenomenon suggesting antibiotic activity has been observed with both these organisms (Beard 1946). When both are present in the soil, only one of them will develop in a single host, depending on the relative concentration of the spores. Their effect on the growth of other organisms has not been investigated.

*Serratia marcescens* Bizio, a red-pigmented bacterium isolated from a dead Japanese beetle grub in 1951, proved to be highly pathogenic when injected into healthy grubs. This bacterium occurs commonly in nature.

*Bacillus alvei* Ches. and Chey., the bacterium associated with European foulbrood of the honey bee, was found to be highly pathogenic to grubs of the Japanese beetle. This disease has not been investigated further for the control of the beetle.

#### RICKETTSIAS

A fatal infection of Japanese beetle grubs caused by *Coxiella popilliae* Dutky, one of the rickettsias, was discovered at Nottingham, Pa., in 1940 (Dutky & Gooden 1952). It has since been encountered in grubs from several widely separated areas, and at some localities large numbers of diseased grubs have been found repeatedly. While most of the recoveries have been made from Japanese beetle grubs, the disease has been diagnosed also in grubs of *Phyllophaga anxia* and *P. ephilida*. This suggests that still other species of Scarabaeidae may be susceptible. Affected grubs have a greenish-blue discoloration of the fat bodies, which led to its designation as blue disease.

Japanese beetle grubs were infected by injecting them with suspensions of diseased blood or with filtrates of these suspensions, and also by introducing them into soil inoculated with such suspensions. The attempts to isolate and culture the rickettsia have not been successful, but the organism has been maintained by serial inoculation of grubs. Preliminary studies indicate that 30° C. (86° F.) is about the upper limit for the development of the disease; the threshold temperature has not been determined. The attempts to colonize this rickettsia in the field on a small scale have been only partially successful.

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## DISCUSSION

J. A. ADAMS. Please explain how the *Tiphia* parasite assists in the distribution of milky disease organisms.

W. E. FLEMING. The disease spores adhere to the body of *Tiphia* when it burrows into infected soil. When the wasp flies to another spot some of these spores are deposited there.

R. E. CAMPBELL. Will the disease affect other grubs?

W. E. FLEMING. Milky disease will affect other Scarabaeidae. With repeated passage of disease through grubs of a given species the potency against that species tends to increase.

C. J. S. FOX. What are the main factors which limit the spread of the *Metarrhizium*?

W. E. FLEMING. Temperature and moisture. When dry conditions prevail, the incidence of disease becomes progressively less. However, the disease remains in a dormant condition.





# Seed Treatment as an Aid to Vegetable Insect Control

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## ABSTRACT

The control of insects attacking the underground portions of crop plants was given a tremendous impetus by the recent introduction of several highly potent insecticides. Usually, these insecticides are applied broadcast or localized near the growing plants. However, there are instances when seed treatment is an easy and effective means of application. This is particularly true with vegetables.

The use of seed treatments or dressings dates back many years when calomel was dusted on seeds of radish and onion for root maggot control. Of recent date, the chlorinated hydrocarbons and organo-phosphorous compounds have been found more effective and positive. Seed dressings on corn, beans, onions, crucifers and other crops are in practice today for control of wireworms and root maggots.

Seed treatment refers to the application of a pesticidal chemical to dry seeds either by soaking the seed in a suitable formulation or sticking the chemical to the seed coats. Such procedures were mentioned in the early writings of economic entomologists searching for a practical means of protecting seeds and seedlings from attack by soil insects. The search was in vain until recent years when with the development of chlorinated and organophosphorous insecticides seed treatment has been elevated to an important place in insect control. At the present writing seed treatment has been most useful for control of root maggots and wireworms. The area of usefulness may be greatly extended in time with the newer systemic insecticides.

Seed treatment is appealing because of the simplicity of application and low cost. The operation is done usually by the seedsman as part of his service in marketing seeds. However, small quantities of seeds may be treated without special equipment by the vegetable grower or home gardener. Materials containing a combination of insecticide and fungicide are offered for sale as specific formulations for seed treating.

Insect control from seed treatment is not as a rule as effective as broadcast applications of the insecticide to the soil or injections into the row at planting time. Moreover, protection by seed treatment may be temporary and unless the current infestation is eliminated later generations will develop unaffected by the treatment. The method is most effective in those instances when the insect pest feeds on the treated seed or in close proximity to it. Wireworms and the seed-corn maggot, *Hylemya cilicrura* (Rond.), come to the germinating seeds soon after planting and are thus exposed to the insecticide. On the other hand infestations of the onion maggot, *Hylemya antiqua* (Meig.), and carrot rust fly, *Psila rosae* (F.), appear weeks after planting, yet seed treatment is effective. This apparent contradiction may be explained on the basis that the larvae feed or move about the area of the spent seed and come in contact with the insecticide that covered the seed coat.

## HISTORY OF SEED TREATMENT

Seed treatment dates to the middle of the last century when entomologists were searching for ways and means of protecting germinating seeds from wireworm attack. Thomas (1930, 1940) in his reviews on wireworm biology and control listed numerous references to seed treatment. Poisons such as the arsenicals and mercurials and substances considered repellants, such as lime, tar and ash, were of no practical value. Thus, control of wireworms by seed treatments was disappointing until the advent of BHC, lindane and other chlorinated insecticides. A number of papers (Lange *et al.* 1949, Kulash 1953) have been published showing favorable results of seed treatments on maize, beans, peas and a variety of vegetable seeds. Some difficulty was experienced with corn in that only a portion of the wireworm population was attracted to the seed. The remainder escaping exposure to the treatment later caused injury to the roots and stems which were out of range of the insecticide. Similarly, root crops such as radish, carrot and parsnip may be injured late in the season by the portion of the wireworm population unaffected by the seed treatment. Although not entirely satisfactory, seed treatment has a place for wireworm control.

Seed treatment for root maggot control is a recent innovation. Two decades ago Glasgow (1934) recorded his experiences with calomel coated onion and cabbage seed. The results of his experiments were mediocre but the simplicity of the application warranted recommendation to growers reluctant to use other means of control. The high cost of mercury compounds was a deterrent to general acceptance of the application. This disadvantage was erased in later years with development of the chlorinated and phosphorous bearing insecticides. Several papers (Ditman *et al.* 1955, Finlayson *et al.* 1954, Peterson 1954) have been published on the success of seed coatings to prevent damage by the seed-corn maggot to beans, peas and other seeds, by the onion maggot and the carrot rust fly.

#### ROOT MAGGOTS OF ECONOMIC IMPORTANCE IN NEW YORK STATE

In New York State root maggots have been more serious pests in the vegetable growing areas than wireworms. The latter when known to be present are effectively controlled by broadcast applications of aldrin, heptachlor, chlordane and dieldrin. These materials when thoroughly mixed with the soil are slow to degradate, hence, are effective for a number of years. Recommendations for wireworm control have favored soil applications rather than seed treatments.

Root maggots have presented serious and individually different problems. The type of application needed for control depends upon the end results desired. Seed treatment will give a short term protection whereas a soil application will protect the crop throughout the growing season.

#### SEED-CORN MAGGOT

The seed-corn maggot, the most common of the root maggots, is a serious pest on beans when conditions are conducive to infestation. Maggots present in the soil at planting time are attracted to the germinating seeds. They feed on the softened cotyledons and the plumule. The latter injury is serious since the loss of the growing point kills the seedling or delays growth. Injury to the cotyledons permits entry of soil-borne disease organisms which breakdown and destroy the vital food supply for the germinating seeds. The sum total of these injuries is an uneven stand of plants or an entire loss of the crop.

Before the advent of seed treatment growers were advised to plant during the pupation period of the first generation; a date that was predetermined for them by the entomologists. This method was not always positive and fields planted during the "maggot-free date" were often severely injured. With seed treatment, planting may be done at the growers' convenience. Entomologists are not in full accord on the role of the maggots in producing poor stands of beans. The problem is complex due to presence of soil-borne decay producing organisms which attack the germinating seeds. Seed treatment is most useful at times when germination is delayed due to wet, cold soil. Since conditions favoring maggot and disease attacks are unpredictable, seed is treated to meet these eventualities should they arise.

Treatment consists of mixing an insecticide, lindane, dieldrin, aldrin or heptachlor and a fungicide in a sticker (4% methyl cellulose solution). This slurry is applied to the dry seeds so that they are evenly coated. Upon drying the mixture adheres tightly to the seed coat. At the time of germination the maggots are attracted to the treated seed and come in contact with the insecticide. The critical time for attack passes with the emergence of the sprout; consequently the period of protection is a short one.

#### ONION MAGGOT

The onion maggot has been a threat to onion production for the past five years. New York's onions are produced largely on the organic or muckland soils. Infestations have been sporadic, appearing serious in some fields of an area and absent in others. Seed treatment is one of two methods used by onion growers to combat the maggot. The other is a row application wherein the insecticide in a weak solution of formaldehyde is injected into the row at planting time. The formaldehyde is a sterilant to suppress soil-borne diseases. The row application or drench requires large supply tanks to be mounted on the seeders and a piping system to conduct the liquid to the seed furrows. This rigging is not adaptable to the small seeder, hence many growers use treated seed since they do not have proper equipment for the drench method. As a rule seed treatment is not as effective as row application.

The seed dressing has two components, the insecticide and a fungicide, in quantities that make for a thick coating. For binding the pesticides to the seed coat a sticker such as

methyle cellulose or gum arabic is needed. The operation proceeds by mixing the insecticide with the sticker. Then the mixture is applied to the dry seeds after which the wetted seeds are shaken vigorously with a fungicide powder of thiram or captan.

The procedure of combining the pesticides may be varied without jeopardizing efficiency. For instance the insecticide and fungicide may be premixed before application to the seed. Or the seeds may be wetted with the sticker followed by addition of the insecticide, then by the fungicide. Vigorous shaking after addition of each powder is necessary to distribute the chemical evenly over each seed. Shakers such as those used to reconstitute paint and found in most paint stores are excellent for the shaking procedure.

Wettable powders of heptachlor, dieldrin, aldrin, parathion and endrin are of equal effectiveness. The rate of application is one pound of toxicant to 100 pounds of seed. Lindane and chlordane are less effective than the foregoing and in some instances are injurious to germination. Some promise is shown for one or two systemic materials.

Wettable powders are used instead of the emulsifiable formulations because the latter are injurious to certain varieties of onion seed. The solvents are responsible for poor germination and evidence indicated that the solvents penetrated the seed coats into the embryos. Varieties such as the standard Early Yellow Globe were seriously affected by xylene and certain aromatic petroleum distillates but Sweet Spanish was exceptionally tolerant of these solvents.

Since maggot attacks follow planting by several weeks it is surprising that seed treatment is effective after such a long period. The answer rests in the feeding habits of the newly hatched larvae. Eggs are laid slightly below the soil surface near the plants. Upon hatching the young maggots migrate downwards to start feeding at the juncture of roots and stems. At this spot is the deposit of insecticide remaining from the spent seed. Only the very young larvae succumb as experiments have shown that large maggots are unaffected by the deposit.

#### CARROT RUST FLY

The carrot rust fly, a pest of carrots and celery grown on mucklands, is as sporadic and unpredictable as the onion maggot. In New York State the pest has two generations and possibly a partial third. The first one injures the taproots of the young carrot plants causing prolonged and misshapen roots. The second generation appears in the late fall season near time for maturing of the crop. The maggots tunnel into the fleshy roots.

Seed treatment is only partially effective as a means of control. Insecticides applied in the same manner as on onion seed have been fully effective against the first generation maggots but totally worthless in suppressing the second brood. Therefore, the crop must be lifted before the second generation maggots appear on the scene. Carrot growers rely on seed treatment only to protect the early crop that is harvested by the first week of September.

The roughened seed coats of carrot will hold heavy deposits of powdered insecticide but a sticker increases the tenacity of the coating to the seed. The actual operation is a slight modification from treating onion seed. Carrot seeds are moistened with enough sticker solution to form a crumbly mass without individual seeds adhering too firmly to each other. The insecticide powder is added and the whole is shaken vigorously until the seeds are evenly coated.

A fungicide may be added to the coating although this is not always necessary except where parathion is used as the insecticide. In many instances stands from parathion coated seed have been poor. Scant evidence indicated that soil-borne diseases may be responsible for the failures. The addition of a fungicide of thiram or captan has enhanced germination. No difficulty with poor stands has been met with the chlorinated insecticides. The most effective of the materials tried have been aldrin, dieldrin, heptachlor, endrin and parathion used at the rate of one pound of toxicant to 10 pounds of seed. Evidence of phytotoxicity was noted with use of the emulsifiable solutions.

Only postulation can be offered as to the way the coating controls the first generation maggots. Apparently the toxicant is deposited in a localized zone around the taproot and near the soil surface. The young larvae after hatching move downward close to the taproot and through the zone of the insecticide. As the roots grow and push outwards the insecticide is scattered until by late fall the concentration is insufficient to be toxic to second generation

larvae. Although control of the first brood as judged by plant growth appears adequate, soil sampling during the pupal period showed a small number of pupae present. Thus while seed treatment reduced the population to numbers too low to cause significant plant damage it failed to eliminate the population. This was a serious disadvantage since the surviving pupae gave rise to a second generation. However, for protection of early crop carrots, seed treatment is effective and convenient to apply.

### SYSTEMIC INSECTICIDES

Attention is being focused on the insecticidal possibilities of the systemic insecticides, chemicals which are readily absorbed by and translocated in the plant. As seed treatments, evidence is mounting that the systemics will control aphids and thrips. Some of the materials show promise for onion maggot control. It is anticipated that the same applications will be of value in suppressing onion thrips populations during the early part of the growing season. A wide area of usefulness may eventually be found for the systemics.

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# A Comparative Appraisal of Wireworm and Seed-Corn Maggot Control in the Western United States<sup>1</sup>

By W. H. LANGE, JR.,<sup>2</sup> M. C. LANE<sup>3</sup>, and M. W. STONE<sup>4</sup>

## ABSTRACT

Wireworms, the active feeding stages of click-beetles (Elateridae), cause damage to most irrigated crops in California, Oregon, Washington, Idaho, northern Utah, and western Montana. Dry land crops, particularly cereals, are also occasionally damaged. These active larvae not only reduce stands of many crops by feeding on the germinating seeds, but also bore into roots, stems, bulbs, or tubers. Being long-lived, they remain in the soil to damage plants over extended periods of time. Wireworms cause extensive losses to potatoes, corn, onions, lettuce, melons, cucumbers, squash, beans, tomatoes, peas, carrots, spinach, sugar beets, sorghum, cereals, and certain flower crops, and few crops are exempt from attack.

Irrigated land wireworms belonging to the genus *Limonius* cause the most extensive losses in most areas. With the advent of DDT and even more potent chlorinated hydrocarbon insecticides, new and better selective fumigants, and signal advances in methods and equipment to apply these chemicals, wireworm losses to large acreages are now a rare occurrence. In California, for example, wireworm losses are now only sustained on new land, on peat or muck soils, old orchards which are disked up and planted to row crops, overflow lands which are periodically cropped, and non-irrigated areas planted to low net income crops.

The present compendium of wireworm and seed-corn maggot control in the western United States is devoted chiefly to wireworms except for a discussion of seed-corn maggot control in California where the insect has now become as important as wireworms as a factor in stand establishment. The present centers of investigations of wireworm control in the western United States divides our subject conveniently into three sections, as follows: 1, "Wireworm and seed-corn maggot control in Northern California", by W. H. Lange, Jr.; 2, "Wireworm control in lima beans in Southern California", by M. W. Stone; and 3, "Control of wireworms in the Pacific Northwest", by M. C. Lane.

## INTRODUCTION

The investigations of Lane were first initiated in 1920 on the Great Basin wireworm in Eastern Washington, and extended to irrigated land wireworms in the Yakima Valley in 1924. The wireworm laboratory at Walla Walla, Washington, was open in 1928 and continued to the present time. Lane's investigations have demonstrated the great value of cultural control in reducing wireworm populations. Practices such as flooding, fall plowing, and crop rotations were found of value. The work in the Pacific Northwest has demonstrated the value of DDT applications when evaluated over extended periods of time. Ten pounds of actual DDT gave control for seven years, with 20 and 40 pound rates still giving complete kill after 10 years. DDT has the advantage of not being absorbed by the plants in detectable amounts, nor imparting off-flavors.

Control investigations by Stone in Southern California were started in 1928 and extend to the present time. Although many trapping methods, flooding, and crop rotations were investigated, none could be effectively used under conditions of continual plant cropping. The use of the fumigants, D-D and ethylene dibromide, gave excellent wireworm control, and with the advent of DDT this chemical was extensively used at the rate of 10 pounds of actual chemical per acre. The application of these soil insecticides has benefited growers of lima beans, sugar beets, tomatoes, potatoes, corn, carrots, and other crops in southern California and made extensive wireworm losses practically a thing of the past.

Investigations on wireworm control in northern California were initiated by Lange in 1942. In 1946 headquarters were moved from Salinas to Davis where work continues. Tests were conducted using the calcium cyanide and bait crop method of Campbell (1924), dichloroethyl ether and other chemicals. A 5-year study was made of crop rotations in relation to wireworm increase in the valley (1943-47). In 1943 tests with the new fumigants

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ethylene dibromide and D-D mixture resulted in the extensive use of the fumigation method in this valley. The residual insecticides BHC, and DDT were first used at Davis in 1946 and these tests were followed to the present time with other chemicals of this nature. The application of chemicals to seeds as seed coatings or dressings have been widely used in California based on work at Davis begun in 1946 and continued until the present time. About 30 different kinds of seeds are being treated at the present time with combination insecticide-fungicide mixtures.

The seed-corn maggot, *Hylemya cilicrura* (Rond.), is generally distributed in the western United States causing damage to germinating seeds of all types. In California the larvae also kill seedlings of alfalfa and onions, bore into the petioles and crowns of celery and spinach, and into emerging asparagus.

Stone (1953) points out that with the widespread use of soil insecticides there has been a noticeable increase in populations of the seed-corn maggot. He demonstrated that the sugar-beet wireworm is predaceous on maggots and that probably with the great reduction in wireworms, maggot survival and resultant increase in damage occurs.

In northern California the seed-corn maggot is now one of our most important pests of germinating beans and other seeds. Most injury is associated with the plowing under of green crops or the application of large amounts of organic fertilizers. Insecticide seed treatments were found to be very effective in reducing seed-corn maggot damage to germinating seeds, and to protect from wireworms at the same time.

## 1. WIREWORM AND SEED-CORN MAGGOT CONTROL IN NORTHERN CALIFORNIA

By W. H. LANGE, JR.

Investigations on wireworm control in Northern California were initiated in 1942 at the request of lettuce growers in the Salinas Valley due to substantial losses caused by the sugar-beet wireworm, *Limonijs californicus* (Mann.). In the Salinas area damage was most severe following several years of alfalfa.

Wireworms in Northern California cause damage to practically all types of plants, but during these investigations economic and often severe losses occurred to lettuce, beans, sugar beets, spinach, carrots, white potatoes, sweet potatoes, Brussels sprouts, corn, milo, onions, garlic, tomatoes, strawflowers, chrysanthemum, barley, wheat, sunflower, Kohlrabi, pine seedlings, and strawberries.

The species of wireworms causing most damage in northern California are the sugar-beet wireworm, *Limonijs californicus* (Mann.), the Pacific Coast wireworm, *L. canus* (Lec.), the western field wireworm, *L. infuscatus* Mots., the Oregon wireworm, *Melanotus oregonensis* (Lec.), and *Anchastus cinereipennis* (Esch.). In addition to these species, occasional outbreaks have occurred of *Limonijs clypeatus* Mots., *Aeolus* spp., *Cardiophorus* spp., and *Melanactes densus* Lec. In 1951 *Ctenicera pruinina* Horn, caused losses to pine seedlings in Lassen National Forest. *L. clypeatus*, although usually considered to be quite a rare species, in 1949 caused a loss of 200 acres of barley in the Davis area on non-irrigated land. The sugar-beet wireworm is the commonest species in coastal areas in Northern California and seems to adapt itself better to heavy clay soils, whereas the western field wireworm prefers sandier soils. *L. canus* is the commonest species in the interior valleys where it is usually associated with lighter soil types. *Anchastus cinereipennis* survives on overflow lands, often flooded all winter, and in the delta area—soils usually rich in organic matter. *Melanotus oregonensis* is often associated with dry land farming, the larvae attacking crops rather late in the spring, in both coastal and interior localities.

### WIREWORM CONTROL INVESTIGATIONS

With the advent of DDT, other new and more potent chlorinated hydrocarbons, effective and economical soil fumigants, together with new methods of applying these materials effectively, extensive damage from wireworms in Northern California is almost a thing of the past. Damage still occurs annually to crops grown on new land, to row crops planted on land formerly devoted to orchards, to crops grown on overflow lands, and to low net income, often dry farmed crops. Along with the use of these new chemicals certain problems have arisen:—the effects of accumulations of soil insecticides on growth, possible translocation of toxic residues into the edible portions of plants, and off-flavors due to

absorption of certain chemicals by plants. Many variable factors determine the effectiveness of the chemicals used, their longevity in the soil, and possible effects on plant growth or quality of product. These include the species of wireworm, populations present, soil type and texture, cropping and tilling practices, rates of chemicals applied, nature of the soil biota, and many others.

#### SALINAS WIREWORM STUDIES

A 5-year study of crop rotations commonly used in the Salinas Valley was made from 1943-47 in a typical lettuce growing area near Salinas in order to determine if rotations are useful; or possible, in reducing *Limoniuss californicus* populations. The presence of a crop cover of vetch or alfalfa during the flight period of the adults was found to result in increased populations of wireworms. Continual cropping to lettuce or other row crops maintained wireworm populations, and none of the possible rotations seemed to offer much hope in greatly reducing populations. The high land values in the Salinas Valley in most instances precluded the use of long fallow periods, or crops of low net income.

In 1942 dichloroethyl ether was used for wireworm control on lettuce with fair results when used at the rate of 20 ml. per gallon with 2 per cent of an emulsifying agent (Tergitol 7) and a total of 600 gallons per acre, applied to small plants. Granular calcium cyanide at the rate of 100 pounds per acre drilled into a bean trap crop, according to the method of Campbell (1924), gave a 60 to 85 per cent reduction of wireworms in the rows. Tests in 1944 using crude naphthalene flakes at the rate of 800 pounds per acre were not satisfactory. Calcium cyanamid at 1000 pounds per acre was not effective.

D-D mixture as a soil fumigant for wireworms was first used in experimental plots in 1943. Four hundred pounds of D-D mixture drilled into the soil at 12-15 inch spacings together with 35 pounds of ammonia per acre, increased the yield in one experiment from 91 crates of lettuce to 450 crates. The D-D alone increased yield to 250, 260, and 272 crates using 200, 400, and 600 pounds. Fields commercially treated during 1943-44 controlled wireworms up to 7 years before re-treatment was necessary. About 600 pounds was needed for 100 per cent kill of wireworms.

Experiments with ethylene dibromide were started in 1945. Its selective action on wireworms and lower cost than D-D mixture made it more generally accepted as a soil treatment. Special application rigs were made at this time to apply EDB and more concentrated forms were used as the application equipment became more precise. A rate of 2 to 4 gallons of actual chemical in a naphtha solvent was usually applied. The results of these early trials are presented by Lange (1945, 1946). Control lasted from one to five years depending upon re-infestation. At the present time it is sold as an 85 per cent solution, used at 3-5 gallons per acre straight or diluted with a petroleum thinner.

Although generally effective under most conditions, the penetration of soil fumigants in heavy soil is limited; as shown by Allen and Raski (1950). In actual practice both D-D mixture and EDB have proved relatively ineffective at the usual rates in soils high in organic matter, such as peats or mucks.

EDB and D-D mixture are also used for nematode control, and in certain areas for a "stimulation" of growth following treatment. Soil fumigants are still widely used in Northern California, particularly where both wireworms and nematodes are a problem in the same fields.

#### DAVIS WIREWORM INVESTIGATIONS

In 1946 and 1947 the first seed and soil treatments for wireworm control conducted with BHC and DDT, indicated the rapidity of kill with BHC and slow action of DDT. The results of a five-year study of BHC and DDT as soil insecticides is given in the article by Lange (1956), together with a summary of other materials as residual soil insecticides.

#### RESIDUAL SOIL INSECTICIDES

Following the initial work on BHC and DDT in 1946-47, trials with toxaphene, lindane, chlordane, and parathion began in 1948, aldrin and dieldrin in 1948, methoxychlor and DDD in 1949, and heptachlor, isodrin, and endrin in 1952. On the basis of these trials

the residual soil insecticides tested can be grouped according to their initial kill and residual activity against *Limoniuss* spp. as follows:

Group 1.—Lindane, BHC, endrin, isodrin. These materials give rapid initial control of wireworms, yet have residual activity. Endrin and isodrin at 3–5 pounds per acre give the same velocity of action as  $\frac{1}{2}$  to 1 pound of lindane.

Group 2.—Aldrin, heptachlor. The velocity of action appears slower than endrin or idodrin, and faster than dieldrin, and have residual properties, but not as much as dieldrin or DDT.

Group 3.—DDT, dieldrin. These chemicals are slower in action than lindane, BHC, isodrin, endrin, aldrin, and heptachlor, and their full effects are best evaluated over a period of years following treatment.

Group 4.—Parathion, chlordane. These chemicals often give excellent immediate control but have limited longevity in the soil.

In addition to the chemicals listed, methoxychlor, DDD, and toxaphene were not effective at the rates tested.

The effective rates of several chemicals and their persistence determined by their biological activity are given in Table I. In many crops it is apparent that an ideal soil insecticide should not only give an initial control and protection during the current season, but also have long-lasting qualities and kill young larvae over a period of several years.

TABLE I—Effective Rates of Certain Soil Insecticides and their Residual Nature in Soil, Northern California, 1947–1956\*.

Insecticide	Effective dosage range per acre (Pounds actual)	Persistence as determined by biological activity (Years)
DDT	10–40	5
Chlordane	5–10	1–2
Parathion	5	$\frac{1}{2}$ –1
Aldrin	2–5	1 $\frac{1}{2}$ –2
Dieldrin	2–5	2–2 $\frac{1}{2}$
Endrin**	2–5	2
Isodrin**	2–5	2
Heptachlor**	2–5	1–2
Benzene hexachloride (technical)	$\frac{1}{4}$ –1 (gamma)	1–2 $\frac{1}{2}$
Lindane	$\frac{1}{4}$ –2	1–3 $\frac{1}{2}$

\*Effective rates refer to an approximate LD-50 point based on laboratory and field studies.

\*\*Residual nature still under investigation.

An intensified attempt to study the effects of insecticidal treatments on possible absorption into the plants, and off-flavors or other degrading effects was made for the 1951–52 plots (Lange, 1956). The 1947 tests with BHC and DDT had indicated off-flavors with BHC at rates of 1 to 2 pounds of gamma isomer in fresh cantaloupes, fresh, canned, cooked, and juiced tomatoes, fresh, cooked, and canned carrots, dry and cooked lima beans, and cooked white potatoes. Later tests indicated no serious residues or off-flavors with DDT, parathion, aldrin, dieldrin, endrin, and heptachlor in root vegetables although carrots, in particular, seemed to take up small amounts of aldrin. Isodrin gave off-flavors in certain vegetables several months after processing. Lindane imparted off-effects to most root vegetables upon processing at rates as low as  $\frac{1}{4}$ –1 pound per acre.

Trials in 1951 by Lange and Carlson indicated that in a normal tillage operation, roto-tilling or multiple disking, over twice as much chemical occurs in the upper 2  $\frac{1}{2}$  inches of soil as the next lower 2  $\frac{1}{2}$  inches.

Residual soil insecticides are being used at the present time in Northern California chiefly for the protection of white and sweet potatoes. Aldrin at 5 pounds per acre is the



usual choice as protection during a single season is required. DDT is effective in heavy soils at 20-40 pounds per acre, although it may be effective in some cases at 10 pounds the first season in certain of our lighter soils. DDT at 180 pounds actual per acre at Salinas in heavy clay loam soil had no observable effect on the growth of lettuce.

SEED TREATMENT FOR WIREWORM CONTROL

About 30 different kinds of seeds are being treated in Northern California for the control of wireworms and seed maggots and pre-emergence damping-off fungi. These combination insecticide-fungicide treatments have proved economical, relatively effective, and safe at the proper dosage levels. The two commonly used insecticides are lindane and dieldrin, although some heptachlor, aldrin and endrin treated seed is planted.

The work in Northern California has proceeded along two lines—one, to determine the amounts of chemicals necessary on seeds to control the insects, and two, the amounts of insecticides the seeds will tolerate. This approach involves greenhouse trials in pasteurized and *Pythium*-infested soil, field trials, and finally commercial use. Seed treatments involving insecticides are economical, ranging from a few cents an acre to \$1.50 an acre. Some of the newer systemic insecticides applied to seeds are more costly. The operation can usually be combined with the application of the fungicide.

Chemicals can be applied to seeds in four ways: 1, as dusts, by means of rotary mixers; 2, as dusts to which water or stickers are sprayed on the seed as it is rotated (liquid fixation

TABLE II—Seed Type and Effective Rates of Lindane for Wireworm Control in Relation to the Amount of Seed Planted, Seeds per Ounce, Kindane per Seed, and Amounts Applied per Acre.

Seed	Ounces Lindane per 100 pounds seed		Pounds Seed Planted per Acre*	Number Seeds per Ounce*	Deposit of Actual Lindane per Seed (milligrams)	Amount Actual Lindane per Acre (ounces)
	75%	100%				
Sugar beets	5.33	4.00	8-12	1,644	0.0431	0.320
Tomato	2.66	2.00	0.5	11,482	0.0039	0.01
Cotton	2.66	2.00	25	226	0.1570	0.50
				(acid delinted)		
Corn	1.33	1.00	6-12	110-120	0.1613	0.12
Peas	1.33	1.00	60-100	85	0.2088	1.0
Okra	1.33	1.00	8	450	0.0394	0.080
Cucumbers	1.33	1.00	1-4	1,077	0.0164	0.04
Cantaloupes	1.33	1.00	1-4	1,276	0.0139	0.04
Watermelon	1.33	1.00	4	312	0.0568	0.04
Lettuce	1.33	1.00	1-1.5	25,175	0.0007	0.015
Carrot	1.33	1.00	3.25	23,417	0.0007	0.0325
Barley	1.33	1.00	80-100	852	0.0208	1.0
Milo	1.33	1.00	2-5	937.5	0.0189	0.05
Sunflower	1.33	1.00	3-7	187.5-562.5:250	0.0710	0.07
Onion	1.33	1.00	4-5	9,667	0.0018	0.04
Spinach	1.33	1.00	8-15	2,835	0.0063	0.08
Beans (baby bush limas)	0.66	0.5	45-55	71	0.1250	0.275
Beans (bush, snap, all others except limas)	0.66	0.5	30-60	113	0.0785	0.30
Oats	0.66	0.5	75-90	795.2	0.0111	0.45
Sudan grass	0.66	0.5	3-15	3,408	0.0026	0.075
Rye	0.66	0.5	80-100	1,136	0.0078	0.50
Safflower	0.66	0.5	30-40	500-812.5:562.5	0.0157	0.20
Alfalfa	0.66	0.5	2-15	14,200	0.0006	0.075
Soybean	0.66	0.5	30	170-369	0.0240	0.15
Radish	0.66	0.5	10	4,000	0.0022	0.05
Cabbage	0.66	0.5	2	8,930	0.0009	0.01
Beans (standard large limas)	0.33	0.25	100-120	23.6	0.1880	0.30
Beans (Fordhook large limas)	0.33	0.25	100-120	28.5	0.1556	0.30
Wheat	0.33	0.25	80-100	710	0.0062	0.25

\*Numbers in italics indicate average amounts or numbers.



method); 3, as slurries, or thick suspensions of chemicals applied in slurry machines; and 4, as solutions or suspensions by means of spray treaters.

Slurry and liquid fixation treaters are available (see Kloor, 1954; Walters, 1954). Dusts are commonly applied to cereals, but are losing favor for the application of insecticides due to the amounts lost in subsequent handling.

Seed treatment began in California in 1946 when BHC and DDT were applied to baby lima beans for garden centipede control and to sugar beets for wireworms. The early history on sugar beets starting in 1947 is given in the article by Lange and Leach (1950). The phytotoxicity of BHC led to extensive tests using lindane (Lange *et al.*, 1949, 1949a, 1950) and lindane has been used with thiram, captan, or Ceresan for many seed types. Dosages vary depending upon seed type, such as outlined in Table II. One company in California alone has treated over 3,000,000 pounds of sugar beet seed with a lindane-fungicide mixture, the seeds being dyed to indicate treatment. This expanded usage was made possible by the development of a new spray treater (Kepner and Leach, 1949). The practical use of the treater is discussed by Kloor (1950, 1951).

The 10-year study of seed-treatments indicates that their effectiveness is determined by a number of factors. These factors can be divided into those independent of the insects, and those dependent on insects, as follows:

#### *Insect Independent*

1. Seed type.
2. Viability of seed.
3. Method of application.
4. Chemical and dosage.
5. Storage period and storage conditions.
6. Compatibilities.
7. Formulation used.
8. Soil and climatic factors.

#### *Insect Dependent*

1. Species of insect.
2. Distribution.
3. Uniformity of brood.
4. Life cycle and seasonal activity.
5. Daily movements in soil.

Space does not permit a discussion of all these factors. Seed treatment is limited by the restricted zone of protection. This is partially off-set in certain seeds such as sugar beets, spinach and cereals where the old seed parts or balls remain in the soil and affect wireworms for several months after the plants emerge. Lindane treated oat seed, for example, loses only 30 to 60 per cent of its effectiveness in 30 days in the soil.

Although lindane has been one of the most effective materials on seeds in actually killing wireworms, the following materials have given satisfactory controls on particular seeds and under particular conditions: dieldrin, aldrin, heptachlor, endrin, isodrin, parathion, diazinon, thimet, and EPN-300. Lindane was found to affect from 85 to 100 per cent of the live, active, large worms in the seed zone and in some cases to reduce the total population of large worms in the soil 43 to 50 per cent. The attractiveness of the seed to wireworms is often important. Better kills were obtained with corn and lima beans, for example, which are very attractive, followed in decreasing order of their selectivity by barley, sugar beets, peas, and tomatoes.

Under conditions of adverse germination, low soil temperatures and excessive moisture, and the presence of the seed decay organism, *Pythium ultimum*, a number of chemicals applied to seeds at the usual rates cause an increase in the severity of seed decay and stand losses under field conditions. If *Pythium* is absent no adverse effects occur. The addition of an adequate fungicide usually nullifies the adverse effects and for this reason fungicides are usually applied with insecticides.

#### OTHER WIREWORM CONTROLS

In addition to the use of fumigants, residual soil insecticides, and seed treatments, several other methods of wireworm control are used in Northern California.

The protection of tomato transplants in muck soils has been obtained by treating the transplant water with lindane at the rate of 2 ounces of actual material per acre. Morgan and Lyons (1950) demonstrated that no off-flavors to fresh and processed tomatoes were obtained with lindane used at this rate.

The use of transplanter water treated with pentachloronitro-benzene (PCNB) and heptachlor, aldrin, or dieldrin for maggot and wireworm control of Brussels sprouts transplants is a recent development. In San Mateo County 400 acres have been planted in the last three years using a new machine and metering device (Akesson and Parks, 1955). For clubroot disease  $2\frac{1}{2}$  to 3 pounds of 75 per cent wettable PCNB is used per 100 gallons of water, and 2 to 4 ounces of 50 per cent wettable insecticide.

The use of insecticides in fertilizer dry mixes is not widely used in Northern California, but aldrin is being used on corn at 5 pounds actual per acre in dry mix fertilizers with good results, particularly in peat soils where general broadcast treatments are not effective.

#### SEED-CORN MAGGOT CONTROL

With the control of wireworms the seed-corn maggot, *Hylemya cilicrura* Rond., has become one of our most important pests of germinating seeds of all kinds. In 1949 investigations began in the Santa Clara Valley on maggot control of Fordhook lima beans planted for green processing. By 1954 practically 100 per cent of the Fordhook lima beans were being treated with a combination of insecticide and fungicide for maggot and wireworm control.

The 1949-50 experiments are given in the article by Lange *et al.* (1951). The results of extensive tests on lima beans and cotton are summarized by Leach *et al.* (1954, 1955, and 1956).

In 1955-56 springtails (*Onychiurus* spp.) also damaged the plumules of germinating lima beans.

Extensive tests have indicated that a number of materials give excellent protection from maggot attack. Lange *et al.* (1956) suggest the use of aldrin, dieldrin, endrin, heptachlor, or isodrin, at the rate of  $\frac{2}{3}$  ounce of 75 per cent material per 100 pounds of seed, and 75 per cent lindane at a  $\frac{1}{3}$  ounce rate—all slurried on the seed, and combined with thiram or captan. In experimental tests many other materials were effective including thimet, Bio-1240, diazinon, and guthion. Springtails were controlled by endrin and isodrin.

In 1954 a trial was run at Davis to determine the relative value of seed treatment, furrow applications at the time of planting, surface treatment, soil treatment, the use of a dieldrin-treated fish meal fertilizer, and combination treatments. Based upon the extent of maggot damage and normal emergence, furrow treatment using  $\frac{1}{2}$  pound of dieldrin per acre plus seed treatment at  $\frac{2}{3}$  ounce of 75 per cent dieldrin gave the greatest protection, but there was no significant difference between this treatment and furrow alone, seed treatment alone, surface plus seed treatment, soil plus seed treatment, or treated fish meal plus seed treatment, using the same rate of dieldrin per acre in each case.

Part of the effectiveness of seed treatments for maggot control depends upon the relative attractiveness of different seeds to the seed-corn maggot and its ability to develop in seeds. In 1955 a series of 10 different seeds were planted in replicated plots to determine their attractiveness to maggots. The results indicate that in this trial Fordhook lima beans were the most attractive, or at least a greater number of flies could survive—probably a combination of both factors—followed in decreasing order by peas, barley, cucumber, sweet corn, cotton, onion, beet, lettuce, and tomato. Not only were different numbers recovered, 0 in tomato to 76 for lima beans (per 25 plants), but the rate of development varied considerably, with maggots developing most rapidly on barley.

In addition to the seed treatment method for seed-corn maggot control, there is some use of furrow treatments in Northern California either using dusters that blow chemicals into the rows, or spray treaters similar to the one described by Elmore (1950). Control is reported to be excellent. Surface applications of aldrin, dieldrin, or endrin are also used by some growers, with variable results.

## 2. WIREWORM CONTROL ON LIMA BEANS IN SOUTHERN CALIFORNIA

By M. W. STONE

The sugar-beet wireworm, *Limonius californicus* Mann., has been a major pest of lima beans in southern California for more than half a century. Graf (1914) described the severe damage done by this wireworm to sugar beets, beans, corn, and root crops in 1911. Campbell (1924) reported on its increasing destructiveness to similar crops and the need for finding a method of control. Since the writer was assigned to this problem in 1928, he has observed

portions of lima bean fields destroyed by this pest every year, and many entire fields have been damaged.

During the last 25 years various methods of control have been tried, including bait traps for wireworms, trapping of adult beetles, flooding, and the use of cover crops, all in large-scale field experiments. None of these methods were very effective. Since 1948, DDT (Stone and Foley 1953) and ethylene-dibromide (Stone 1949) have been used extensively in this area. D-D mixture (Stone 1949) has been used to a lesser extent mostly for the control of root-knot nematodes. These materials have been responsible for substantially increased yields of lima beans. Orange and Ventura Counties grow annually over 55,000 acres of dry lima beans (73 percent of the dry large lima bean crop in southern California), and over 8,700 acres of green Fordhook lima beans for processing. The effect of these soil insecticides on wireworms and on lima bean production in these counties during the last 7 years is herein discussed.

With the discovery of effective soil fumigants there was immediate need of equipment for applying these chemicals. The Shell Chemical Corporation and the Dow Chemical Company made the original machines and later local blacksmiths and farmers constructed their own machines. Scores of fumigant applicators capable of treating swaths from 6 to 16 feet wide, either tractor-mounted or trailer types, are now being used in this area.

Commercial operators report the quantities of chemicals used for each pest and the acreage treated to the county agricultural commissioners, who compile and include this information in their annual reports (Table III). At least 10 to 15 percent additional acres are treated annually by farmers who have constructed their own applicators or have applied DDT with their own spray or dust equipment.

Experiments with D-D mixture for the control of wireworms were conducted from 1943 to 1946. It was applied commercially in 1947, at the rate of 300 to 400 pounds per acre. Of the land planted to field crops, a total of 7,553 acres have been treated with D-D mixture, at an average rate of 222 pounds per acre (Table III).

TABLE III—Acreage Treated by Commercial Operators, and Cost of Soil Insecticides used Against Wireworms and Nematodes in Ventura and Orange Counties, 1948–54.

Year	D-D Mixture (222 lb. per acre)			Ethylene dibromide 83% by weight (3.74 gallons per acre)			DDT, 50% WP, (20 lbs. per acre)			ALL MATERIALS	
	Acres Treated		Cost applied*	Acres treated		Cost applied**	Acres treated		Cost applied ***	Acres treated	Cost applied
	Ventura County	Orange County		Ventura County	Orange County		Ventura County	Orange County			
1948	1,665	780	\$118,980	3,250	5,698	\$ 192,611	6,687	—	\$ 56,839	18,080	\$ 368,230
1949	661	49	28,370	6,071	4,776	233,488	3,595	—	30,557	15,152	292,120
1950	708	18	31,674	9,390	1,295	230,011	3,468	200	31,178	15,079	292,269
1951	415	114	20,087	8,819	2,575	245,265	681	712	11,840	13,316	277,421
1952	1,170	35	41,278	8,637	3,519	261,668	—	115	997	13,476	303,751
1953	887	43	33,725	6,870	1,752	185,595	—	—	—	9,552	219,942
1954	958	50	33,698	8,395	3,350	252,821	—	—	—	12,753	288,460
Total	6,464	1,089	\$307,812	51,432	22,965	\$1,601,459	14,431	1,027	\$131,391	97,408	\$2,040,263

\*17c. per pound plus \$3 per acre for application.  
\*\*\$4.90 per gallon plus \$3.20 per acre for application.  
\*\*\*\$8.50 per acre for material and application.  
\*\*\*\*Average cost of all treatments, \$20.95 per acre.

Our experiments with ethylene dibromide were begun in 1945, and following extensive tests in 1946 this material was used commercially in 1947. Close to 9,000 acres were treated in Ventura and Orange Counties in 1948. Originally it was applied in a 10-percent solution, then at 42 percent, at the rate of 10 gallons (2 pounds) of ethylene dibromide per acre. With an improvement in equipment it was possible to use a solution containing 83 percent by weight. This concentration is being applied generally at rates varying from 3 gallons per acre for wireworm control to as high as 6 gallons per acre on land where nematodes are a serious problem. Ethylene dibromide has been used more extensively than D-D mixture because it is cheaper, and because of its effectiveness against both wireworms and nematodes, the latter being a serious problem in fields undergoing continuous cropping. Since 1948 about 74,400 acres of land in Ventura and Orange Counties have been treated with ethylene dibromide at an average rate of 3.74 gallons per acre. The cost to the growers for material and application was approximately \$1,600,000 (Table III).

Experiments with DDT for the control of wireworms were begun in 1945 in field plots, and with the promise shown a small acreage in Ventura County was treated in 1947. Over 6,600 acres were treated in 1948 at the rate of 10 pounds per acre, and this dosage has been generally used since then. No injury has been caused to lima beans, potatoes, onions, carrots, table beets, sugar beets, cabbage, cauliflower, peas, peppers, tomatoes, lettuce and celery in experimental plots, and there has been no off-flavor in the edible parts of any of these crops. Only small amounts of DDT were used in Ventura County after 1951 since most of the badly infested fields had been treated, and the DDT is effective for at least 5 years (Table III). Moreover, now that lindane and other materials are being used to treat the seed there is less need for further treatment of the soil.

DDT was never used extensively in Orange County because growers there were more nematode-conscious and preferred fumigants.

Wireworms can be controlled with DDT at a cost of only \$8.50 per acre for material and application. Owing to its long residual effectiveness, a single treatment would actually amount to only \$1.70 per year, whereas if a fumigant is applied, the yearly cost would be \$21.00 or \$10.50 per acre, because annual or biennial applications would be necessary.

TABLE IV—Acreage Planted to Green Fordhook and Dry Lima Beans, Yields per Acre, and Percent of Acreage Treated Annually with Soil Insecticides, Ventura and Orange Counties, 1948-54.

Year	VENTURA COUNTY					ORANGE COUNTY				
	Acres of		Percent of acreage treated	Yield per acre		Acres of		Percent of acreage treated	Yield per acre	
	Dry beans	Green beans		100-lb. sacks of dry beans	Tons of green beans	Dry beans	Green beans		100-lb. sacks of dry beans	Tons of green beans
1948	32,395	5,286	30.8	19.08	1.58	21,645	4,040	25.2	19.78	1.30
1949	35,082	4,544	26.1	18.18	1.75	29,905	2,400	14.9	17.55	1.38
1950	29,271	4,444	40.2	23.06	1.93	25,000	2,540	5.5	19.12	1.57
1951	28,970	5,812	28.5	22.40	1.88	28,850	2,639	10.8	15.49	1.55
1952	31,108	5,795	26.6	22.52	1.94	24,090	2,080	14.0	20.39	1.68
1953	25,500	7,893	23.2	22.23	1.93	22,765	2,840	7.0	17.42	1.29
1954	27,273	8,518	26.1	21.00	1.82	25,850	2,456	12.0	15.95	1.66
Total	209,599	42,292	—	—	—	178,105	18,995	—	—	—
Average	29,943	6,042	28.7	21.21	1.83	25,443	2,714	12.7	17.95	1.49
Average 1938-47			—	14.23	1.26*	—	—	—	14.46	1.01*

ge 1940-47.



In Ventura and Orange Counties about 64,000 acres are planted annually to large limas or green Fordhook beans (Table IV). At the close of the 1954 season 97,408 acres had been treated with soil insecticides, at a total cost to the growers of \$2,040,662 (Table III).

Ventura County, with nearly three times the treated acreage of Orange County, has received the greater benefit from the application of soil insecticides. The productivity of the soil in the two counties is very similar, and growers follow the same general farm practices in the production of lima beans. In the 10-year period prior to 1948 Ventura and Orange County growers averaged about fourteen 100-pound sacks of dry beans per acre. During the period 1948 to 1954, however, with the greater use of soil insecticides in Ventura County, yields of dry beans have increased every year for an average gain of 7 sacks per acre, as compared to an increase of 3.5 sacks in Orange County. The average price was \$11.47 per 100-pound sack, and bean growers in Ventura County increased the return from each acre by \$58.59, and those in Orange County \$20.71 (Table V). Increases in yield in Orange County in 1951 and 1954 were not sufficient to cover the cost of the soil treatments, and this helped to lower the monetary gain per acre. Judging by the similarity of the yields in the two counties prior to 1948, it appears that the major cause of this increase was due to the use of soil insecticides rather than to improved cultural practices.

TABLE V—Effect of Soil Insecticides on Production of Dry Lima Beans and Green Fordhook Lima Beans in Ventura and Orange Counties, 1948–54.

Year	DRY LIMA BEANS					GREEN FORDHOOK LIMA BEANS				
	Increase in yield (100-lb. sacks) over 10-year average		Price per sack	Net monetary increase or decrease per acre*		Increase in yield (tons) over 8-year average		Price per ton	Net monetary inc per acre*	
	Ventura County	Orange County		Ventura County	Orange County	Ventura County	Orange County		Ventura County	Orange County
1948	4.85	5.32	\$15.96	\$56.46	\$63.96	0.32	0.29	\$189	\$39.53	\$33.53
1949	3.95	3.09	9.02	14.68	6.92	.49	.37	150	52.55	34.55
1950	8.83	4.66	10.83	74.68	29.52	.67	.56	144	75.53	59.53
1951	8.17	1.03	11.14	70.06	-9.48	.62	.54	152	73.29	61.29
1952	8.29	5.93	11.37	73.31	46.47	.68	.67	150	81.05	79.05
1953	8.00	2.96	11.40	70.25	12.79	.67	.28	149	78.88	20.88
1954	6.77	1.49	10.58	50.68	-5.19	.56	.65	148	61.93	75.93
Average	6.98	3.50	11.47	58.59	20.71	.57	.48	155	66.10	52.10

\*Less cost of soil treatment, \$20.95 per acre.

Growers of green Fordhook beans for processing, who have used soil insecticides, have consistently obtained higher yields every year since 1948 when compared with the average in the previous 8 years (Tables IV and V). The average increase in Orange County was 0.48 ton per acre, and benefited the growers to the amount of \$52.11 per acre annually, as compared with 0.57 ton and \$66.10 per acre increases in Ventura County. These and similar gains to growers of sugar beets, tomatoes, potatoes, corn, carrots, and other crops susceptible to wireworm and nematode attack show clearly the benefits from the use of insecticides for the control of soil pests.

Occasionally we receive reports and observe wireworm damage to potatoes and other root crops in soils treated with insecticides. These failures are difficult to diagnose, but are usually due to disregard of soil texture, temperature, or moisture, and failure to observe dosage requirements of different types of soil, as well as to faulty application. Experiments are under way to determine the cause of excess rotting of Fordhook lima beans in some areas



following the application of a soil fumigant. Such problems are bound to arise, but are minor when compared with the over-all benefits. Control is so successful that it is now difficult to find suitable infested fields for use in testing new insecticides for wireworm experiments. Light infestations may be found on land formerly occupied by citrus or walnut trees or planted to alfalfa for several years, but this soil is immediately treated by the growers if used for the production of vegetables or field crops.

In experiments conducted in Orange County between 1933 and 1937, 2.1 wireworms were recovered per foot of row. In the course of soil-insecticide tests in Ventura County prior to 1948, there were as many as 3.4 wireworms per foot of row in the control plots in some years. In contrast, populations in untreated plots in our experiments in Orange County between 1952 and 1955 have never exceeded 0.9 wireworm per foot of row. This reduction in wireworm populations in the soil has had a marked effect on the adult populations. Prior to 1948 many adults could be collected by sweeping alfalfa, but now they are very rarely taken on this crop. With the development of even more effective soil fumigants and insecticides, and with the increased use of seed-coating materials, wireworms in southern California can now be economically controlled.

### 3. CONTROL OF WIREWORMS IN THE PACIFIC NORTHWEST

By M. C. LANE

The first research on the life history and control of wireworms in the Pacific Northwest was conducted in the dry-land areas of eastern Washington about 1910 (Hyslop, 1915). The author began his studies on the Great Basin wireworm in the eastern Washington in 1920 (Lane, 1931, 1935). The research on the biology and control of irrigated-land wireworms was begun in 1924 in the Yakima Valley. In 1928 it was transferred to Walla Walla where it has been continued since (Lane and Stone, 1954). This paper discusses the control of several irrigated or wet-land species of the genus *Limonius*, including the sugar-beet wireworm, *L. californicus*, the Pacific Coast wireworm, *L. canus*, the western field wireworm, *L. infuscatus*, and the Columbia Basin wireworm, *L. subauratus*.

One or more of these species are present in destructive numbers on nearly all the irrigated lands of Washington, Oregon, and Idaho as well as northern Utah and western Montana. They also occur and frequently do damage to cultivated crops in the Puget Sound area of Washington and British Columbia, the Willamette Valley of Oregon, as well as in other farm lands west of the Cascade Mountains. They are not specific in their feeding habits, but attack most all of the garden and vegetable crops grown, either destroying the seed and seedlings or scarring the bulbs, roots, and tubers to reduce their quality.

#### CULTURAL CONTROL

During the early years (1928-1941) of the wireworm investigations at Walla Walla, when chemicals for the control of wireworms were few and very expensive, there was considerable research on control by cultural methods. Laboratory and field studies soon showed that moisture was probably the most important limiting factor in wireworm ability to survive in the soil (Jones, 1937, 1951; Jones and Shirck, 1942). Food was only a factor in the early stages of growth, as after a few months the young larvae could go without food for long periods. Temperature was important in pupation, limiting distribution of these wireworms to areas where midsummer soil temperatures are higher than 68°F. at 6-inch depth. Wireworms move deep enough in the soil in either winter or summer to escape harmful surface temperatures.

While wireworms require a soil atmosphere of 100 per cent humidity, too much moisture can be very detrimental to them in the larval stage, especially in the first year after hatching. Flooding of infested soil (Lane and Jones, 1936; Jones, 1951) in hot summer weather when soil temperatures under the water can be maintained above 68°F. will practically eliminate wireworms after three days. This did not prove to be a practical control for several reasons, mainly because the water leached out the alkali salts, which adversely affected subsequent crops, and flooding could only be practiced on level land.

It was found that these species of wireworms were unable to withstand desiccation. To avoid the surface drying of soil, wireworms will move downward to the damper layers (Jones and Shirck, 1942). When the whole plow depth of the soil dries out gradually, they cannot escape and their numbers will be materially reduced. In order to dry out the soil

deep enough to kill wireworms it is necessary that a crop such as fall grain or alfalfa be grown with a minimum of irrigation water. In clay-loam soil, alfalfa should not be irrigated the last season before it is plowed under to plant truck crops. If the soil is of a sandy-loam type and well drained, it may be possible to irrigate alfalfa early in the season and still dry out the soil sufficiently before the end of the season to kill most of the wireworms. Any financial loss due to reduced yield of alfalfa will be partially compensated for in the increased yield or quality of the next crop.

Wireworms in the pupal stage are soft and easily damaged, and are unable to survive if exposed to the elements during this stage which occurs in July and August. Under irrigation, opportunity often occurs for plowing in summer with subsequent damage to the pupae and reduction in numbers of adults that might emerge to lay their eggs for the following year (Shirck, 1936).

Studies on crop rotations and cultural practices in Idaho and Washington have shown that wireworm populations tended to increase immediately following the growing of red clover and to a lesser extent of sweetclover (Shirck and Lanchester, 1936; Shirck, 1945). Clovers are commonly seeded with small grains, and late summer irrigations are required to keep the young clover plants growing. As small grain crops, such as barley and wheat, are favorable host plants for wireworms, they should be seeded alone so the land can be dried out after harvest. Clovers should not be grown in rotations where wireworms are known to be present unless some method of controlling them is used.

Alfalfa is the key crop in a rotation program to reduce wireworm damage to truck crops (Shirck, 1945), provided the alfalfa is seeded alone and not in a nurse crop of small grain. Wireworms decrease in numbers with each succeeding year of alfalfa, and are further reduced by withholding of irrigation water the last season before planting susceptible truck crops. Potatoes, being very susceptible to wireworm damage, are best grown immediately following alfalfa, and sugar beets next. Such crops as corn and beans should follow these crops. The growing of potatoes in short rotations with grain or clover is not recommended, because large numbers of wireworm adults are produced which deposit eggs under ideal conditions for the survival of a new brood. The growing of row crops continuously in the same soil will usually increase wireworm numbers, necessitating the use of chemical or cultural control methods.

Sod-forming crops, such as pasture grasses without clovers, also help to reduce wireworm numbers in the irrigated lands of the Pacific Northwest (Shirck, 1945).

#### CHEMICAL CONTROL

The control of wireworms with insecticides in the Pacific Northwest was first tried against the irrigated land species in the Yakima Valley in 1924 and in the Walla Walla Valley in 1926. Experiments with arsenicals and other chemicals on seed and in baits did not produce practical methods of control (Lehman, 1932, 1933; Woodworth, 1938). At that time certain of the fumigants seemed to offer the only chance of chemical control where infestations were serious enough to prevent the growing of cash or row crops essential to the economy of irrigation farming (Lehman, 1933a, 1942).

In 1924 calcium cyanide was applied to wireworm infested fields in the furrows at time of plowing (Campbell, 1924). This method was partially effective at a cost of \$30 per acre, but was not generally accepted by the farmers. In 1927 carbon disulfide was tested and dosages were worked out, but the cost was too high for general use (Lane and Gibson, 1932). Because of its cheapness (3 cents per pound) crude naphthalene, a byproduct of gas plants using crude petroleum, was tested as a soil fumigant against wireworms in 1933 (Lane and Stone, 1954). Crude naphthalene flakes, when thoroughly mixed with the soil, gave fair control at rates of application as low as 300 pounds per acre at a cost of \$9 per acre. With the advent of war conditions in the late 1930's, the price of naphthalene increased to a point where it was no longer economical for wireworm control. In the early 1940's three new fumigants came into use for soil pests, dichloronitroethane (ethide), dichloropropane-dichloropropylene (D-D), and ethylene dibromide (Woodworth, 1943; Cook, 1949). These materials all proved to be effective against wireworms and the last two materials are still used successfully against wireworms, nematodes, and other soil pests. Various mechanical means were devised for their application in accurate amounts as well as at specified distances and depths. Cost of application ranged from \$15 to \$30 per acre, depending on soil condi-

tions, which made them rather expensive for use against wireworms alone (Lane and Latta, 1952).

DDT was tested against wireworms in the laboratory at Walla Walla late in 1943, and in outdoor soil cages in 1944 (Lane *et al.*, 1948). DDT kills wireworms by contact and although slow in its action is quite effective, especially against the newly hatched wireworms. Apparently the DDT changes the moisture balance of the wireworms in the soil, as the effect is as though they become desiccated. It is not repellent and seems to be cumulative in its action. Although economical dosages of DDT kill wireworms very slowly, their long life cycle in the soil makes it possible to control infestations over a period of months or a season.

In the spring of 1945 field plots were treated at rates of 10, 20, and 40 pounds of DDT per acre (Lane *et al.*, 1948). This insecticide was broadcast in the form of a 10-percent dust in pyrophyllite. Half the amount was applied to the soil surface and the soil was disk-harrowed and plowed, then the other half was applied followed by another cultivation with disk harrow, to give a thorough mix of the DDT with the soil to a depth of 9 inches. Ten vegetable crops—lettuce, peas, beans, carrots, beets, spinach, tomatoes, cabbage and onions, and potatoes—were grown in each plot, the tomatoes, cabbage and onions being transplants. The growth and yield of these crops were not affected by the DDT. The reduction in wireworm numbers at the end of the 1945 season was 80, 96, and 97 percent in the plots treated at the respective dosages and there was a further reduction in 1946. A test in 1946 with 5 pounds per acre showed that this amount was not sufficient to give a good control. Peas and potatoes were successfully grown for several years in these plots. Chemical analyses were made of various vegetables grown in these plots to determine if DDT was absorbed into their tissues, but the results were negative, except for less than 1 ppm in the potato peelings tested separately. No off-flavors have ever been detected in vegetables grown in DDT treated soil.

DDT was found to have no effect on the wireworm eggs and pupae in the soil, and not enough on the adults to prevent the emerging female beetles from mating and laying their eggs in the soil of the treated plots. Laboratory and field tests showed a definite effect on the newly hatched wireworms, 100 percent being killed in a week's exposure to soil treated with 10 pounds or more of DDT per acre. This high susceptibility of young wireworms to DDT combined with its long residual action in the soil offers one of the best controls for wireworms ever discovered. The original 1945 DDT plots as well as others have been tested each year by placing composite samples of soil from them in pots or cans and then introducing wireworm eggs about ready to hatch to give up to 60 days' exposure of newly hatched wireworms. The 10-pound-per-acre dosage began to show a decrease in toxicity after 7 years, but the 20 and 40 pound rates still give complete kill of young wireworms after 10 years.

Similar effectiveness was found in large commercial fields treated with 10 pounds of DDT per acre from 1947 to 1950. Soil samples taken from some of these fields in 1955 showed a loss of toxicity to wireworms, but most of the fields showed sufficient DDT remaining to prevent reinfestation. This long-term residual action of DDT in the soil to control wireworms and prevent reinfestations, combined with its freedom from phytotoxicity and cheapness at about \$6 per acre for 20 pounds of 50-percent wettable powder formulation, is its principal advantage over most other available soil insecticides. It can be sprayed, dusted, or placed in fertilizers for distribution on the land, and mixed with the soil by ordinary plowing and disk harrowing.

As they became available other insecticides were tested in the laboratory and field against wireworms (Lane and Stone, 1954). Chlordane and BHC were first tested in 1946, toxaphene in 1947, parathion in 1948, aldrin and dieldrin in 1949, heptachlor in 1952, and endrin and isodrin in 1953. Laboratory and statistical methods were developed for comparing these insecticides to give an idea of their toxicity and possible practical use in the field (Lanchester, 1951). All of these insecticides gave good control of wireworms when thoroughly mixed with the soil, but none of them showed the capacity of DDT to remain in the soil and prevent reinfestations of wireworms for several years. BHC and lindane, while very toxic to wireworms in relatively small doses (1 pound or less per acre of the gamma isomer) have a tendency to impart an undesirable flavor to root crops, such as potatoes, carrots, and beets, and therefore cannot be recommended for use on crops to be grown for



human food. Chlordane is erratic in its toxicity to wireworms and more than 5 pounds per acre is necessary to give satisfactory control in most soils of the Pacific Northwest. Aldrin has been used rather extensively for wireworm control, but at the rate of 3 pounds per acre good control has not always resulted. Dieldrin is effective at 2 to 4 pounds per acre. Heptachlor at 2 to 4 pounds per acre gives satisfactory wireworm control, but has a tendency to be absorbed by carrots and parsnips although most truck crops do not seem to absorb it. So far as our experiments have gone, none of these materials except DDT seem to last more than three years in the soil in sufficient strength to prevent wireworm reinfestation, although heptachlor and dieldrin have not been tested long enough to determine this point.

Some seed treating of dry-land wheat to control wireworm damage has been done in the Pacific Northwest (Nelson *et al.*, 1956). Aldrin, heptachlor, dieldrin, and lindane were tested by adding them to the slurry treatment for smut control. The first two materials proved to be equally effective in preventing damage to fall treated seed in the spring. Lindane has a tendency to retard germination if not used very carefully to prevent overdosing. Results have not always been satisfactory because wireworms in the spring can attack the tender wheat plants near the surface above the treated seeds and thus escape contact with the insecticide. The same is true of corn treated for wireworm damage to seed and young plants. Seed treatment has not been extensively used except on dry-land areas, mainly because most truck crop infested lands have already been broadcast treated for wireworm control.

It is estimated that at least 10 percent of the irrigated land in the Pacific Northwest, amounting to about  $\frac{1}{2}$  million acres, has been treated with soil insecticides for the control of wireworms. In southern Idaho alone about 50,000 acres has been treated with DDT in the last eight years, and a considerable acreage was fumigated with ethylene dibromide before 1950. In the Yakima Valley the extremely heavy applications of DDT for control of aphids on potatoes have apparently also affected the wireworm populations, as there is now little demand for their control on land that was once heavily infested. Some of the newer irrigated lands of the Columbia Basin Irrigation Project have been treated to rid the soil of the Great Basin wireworm, *Ctenicera pruinina noxia* (Hyslop), which is always present for the first few years after sage brush or older abandoned lands are put under irrigation. This species does not last long under continuous irrigation, being replaced gradually by the irrigated land species (*Limonius* spp.). A proper attention to rotations and the careful use of soil insecticides when wireworms first appear will keep these pests from doing the damage that has occurred in the past.

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# Chemical Seed Treatments and Cultural Methods in the Control of Wireworms in Field Crops<sup>1</sup>

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## ABSTRACT

*The development of effective chemical seed treatments for the control of wireworms constitutes one of the most important recent advances in wireworm research. Most experimental investigation of seed treatments has been concerned with their phytotoxicity and immediate reduction of wireworm damage. Very little is known about how they affect wireworms. The data from several years' wireworm seed treatment control tests in the Prairie Provinces indicate that more fundamental studies are now necessary, particularly on the relationship between wireworm feeding behaviour and treatment effectiveness.*

*Most wireworm cultural controls are ordinary cultural practices manipulated to reduce wireworm damage. Some appear only to protect the crop and to influence wireworm behaviour rather than survival. Others reduce wireworm populations and maintain them below economically important levels. The extent to which cultural controls for a wireworm species may be initiated and developed is dependent upon what is known about the wireworm itself. Thus, the investigator must be concerned primarily with the effects of environmental factors upon wireworm behaviour and survival.*

*The application of seed treatments and cultural controls together is recommended for the control of wireworms in grain fields in Western Canada. Further studies of the wireworm species involved are necessary to effect their improvement.*

Wireworms, the larvae of Elateridae or "click" beetles, are well-known pests of agricultural crops in many countries. The economically important species feed in the soil upon the seeds, roots, and stems of plants, sometimes for several years before they reach maturity. Damage varies with species, population density, crop, and soil conditions. Although wireworm populations in field crops can be reduced to low levels and maintained there, with chemical and cultural controls, they generally remain a chronic problem in any field where they once become established.

The development of effective chemical seed treatments for wireworm control constitutes one of the most important recent advances in wireworm research; the application of a small amount of insecticide to the seed coat is a simple and relatively inexpensive treatment, and has effectively reduced wireworm damage to a wide variety of field crops. The chlorinated hydrocarbons have been found to be generally more suitable for seed treatments than any other group of insecticides, and of these, aldrin, dieldrin, benzene hexachloride, and heptachlor probably have been tested and used most extensively.

Most of the published work on wireworm seed treatments deals mainly with their phytotoxicity and immediate reduction of wireworm damage and populations. Tests with different species of wireworms under different soil conditions and with different crops have not always provided similar results, so that there are controversial opinions concerning the relative merits of various chemicals used as seed treatments. The primary objective here is not, however, to compare proprietary seed treatments for the purpose of suggesting that any one is more or less useful than another; instead, it is to discuss the present general knowledge of how seed treatments affect plant development and wireworm damage and populations in field crops, and the problems which available evidence suggests require further study.

Any chemical potentially effective as a wireworm seed treatment must be examined in the light of its phytotoxicity before its usefulness as a seed treatment can properly be established. There are various references to the detrimental effects of benzene hexachloride seed treatments upon plant development (Apple, 1954; Hocking, 1950; Kostoff, 1948; McLeod, 1947; Tasker, 1949; Wurgler *et al.*, 1954). In general, external symptoms of phytotoxicity have included swollen and shortened roots and coleoptiles, in varying stages of severity, sometimes followed by a failure of the affected plants to emerge; Kostoff (1948) and Tasker

<sup>1</sup>Contribution No. 3570, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

(1949) showed that these symptoms were caused by incomplete mitotic division of cells, in meristemic tissue, which produced either nuclei with more than the original number of chromosomes, or multinucleate cells. The identity of the chemical responsible for this phenomenon has not been clearly established, however. Hocking (1950) concluded that the plant deformation is not due to the gamma isomer of BHC as such, or definitely to any other major constituent of BHC, although a mixture of trichlorobenzenes prepared by alkaline breakdown of alpha BHC was particularly active; he suggested that BHC preparations for cereal seed treatment should be materials of high gamma isomer content. Conversely, Wurgler, Staehelin, and Bolay (1954), working with BHC seed treatments on wheat, barley, beets, and beans, concluded that the phytotoxicity of BHC is due mainly to the gamma isomer. Benzene hexachloride appears not to be the only offender in this respect, however. Tasker (1949) observed that high concentrations of parathion seed treatments affected cell division and reduced root and coleoptile length, although no hypertrophy was evident. In recent emergence tests at the Entomology Laboratory, Canada Agriculture, Saskatoon, aldrin and dieldrin seed treatments have produced the same external symptoms on wheat seedlings as those described for BHC, although the effects were much less severe.

There is some evidence that environment significantly influences the phytotoxicity of seed treatments. It has been observed that BHC damage is greater in light soils than in heavy soils (Tasker, 1949), that it is not always similar in magnitude in greenhouse and field tests (Apple, 1954; Tasker, 1949), and that it is aggravated by high temperatures (Wurgler, *et al.*, 1954); Hocking (1950) stated that BHC damage is likely to be increased by storage of seed after treatment, especially if ventilation is inadequate, by exposure to sunlight, and by deficient moisture and high pH of the soil. Emergence tests at Saskatoon, with aldrin, dieldrin, and gamma BHC seed treatments on wheat, have indicated that phytotoxicity is greater in sand than in loam soils, greater in greenhouse tests than in field tests, and that organic mercury fungicides used in combination with the insecticide increase the phytotoxic effect of gamma BHC, but not that of aldrin or dieldrin.

It is apparent, then, that a variety of factors may influence the phytotoxicity of chemical seed treatments, although what the toxic active substances are, and how environment affects their activity, have not been clearly determined. If we know just why wireworm seed treatments are phytotoxic, we can reasonably hope that the hazard can be reduced by modifications of formulations; certainly the knowledge will provide investigators with a better basis for selecting, testing, and comparing seed treatments.

While it is generally recognized that seed treatments have provided good protection from wireworm damage to many susceptible crops, there are data which indicate that they do not always affect wireworm damage and populations in the same way, and to the same extent. Lilly (1955, 1956) has reported that aldrin, dieldrin, and gamma BHC appear to repel wireworms from treated corn seed, but that the larvae later attack and kill the developing seedling stems. Faber (1951) obtained good protection, but little reduction of wireworm populations, with BHC seed treatments on wheat. Hampson (1955) stated that trials in Britain indicate dual-action seed dressings appear to initially repel wireworms, leading them to look for food other than the protected seed or seedling. At Saskatoon, preliminary data indicate that aldrin and gamma BHC seed treatments have at least a temporary repelling effect, but in most of our field control tests, aldrin, dieldrin, and gamma BHC treatments have reduced wireworm populations by at least 50 per cent. Hastings and Cowan (1954) also have reported significant reduction of wireworm population with gamma BHC seed treatment.

The implications of apparent repellancy by seed treatments can be discussed more adequately after some consideration of how seed treatments inactivate or kill wireworms. Conceivably, wireworms may be adversely affected by feeding on plant stems, because of translocation of toxicant from the treated seed, by a fumigant effect of the treatment, or by coming in contact with or feeding upon the treated seed. Lilly's work with seed treatments on corn suggests that wireworms are not significantly affected by a translocation of toxicant from the seed to the developing stem. In the Prairie Provinces, we have observed that wireworms frequently cause serious damage to deep-seeded plants, apparently because the larvae feed above the treated seed and are not affected by the toxicant. Although Fredericksen and Lilly (1955) have suggested that the fumigant action of aldrin, dieldrin, heptachlor, and lindane soil treatments can kill wireworms in adjacent untreated soil,

there is no evidence that seed treatments have sufficient fumigant action to affect wireworms in the field. Obviously, the influence of insecticide vapour is determined by its concentration and the susceptibility of the wireworm larvae to it, but we may only speculate now upon the part it plays in repellancy, inactivation, and mortality of wireworms. It is probably safe to assume by inference that wireworms killed by seed treatments are killed mainly by coming in contact with or feeding upon the treated seed; if so, a significant repellent effect, that allows the wireworms later to attack and kill the developing stems, is undesirable, since it does not allow the insecticide to protect the plants or to kill the larvae.

Repellancy alone cannot account for the good protection, accompanied by little reduction of wireworm populations, that has been reported (Faber, 1951), since later damage to the stems would be expected. Dogger and Lilly (1949) have suggested that population density and alternate food plants influence the likelihood of wireworms feeding on treated seed, even though they are initially repelled; that is, the amount of feeding on treated seed is determined by an interaction between the "need" for the larvae to feed on the seed and the repellancy of the treatment, and on this basis we might expect seed treatment efficacy to be influenced by any factor affecting feeding activity. Some differences between the damage/wireworm-population-reduction ratios of different seed treatments, observed at Saskatoon, indicated that a seed treatment may sub-lethally inactivate larvae, so that they cease to feed at least until the plants can survive attack. Finally there is the hypothesis, without supporting evidence, that if developing plants attract wireworms, seed treatments might affect feeding behaviour either by masking the influence of the attractants or by affecting the ability of the larvae to detect them.

In summary, we may logically assume that the amount of wireworm population reduction caused by a seed treatment is dependent primarily upon the feeding activity of the larvae during the time that sufficient toxicant is present in or on the plant. There is evidence that seed treatment repellancy may, under some conditions, significantly reduce the effectiveness of a treatment, suggesting that any non-lethal or sub-lethal influence of a seed treatment upon wireworm feeding behaviour may play an important part in the effect of a treatment upon wireworm damage and populations. Hence, it is apparent that wireworm seed treatment studies must be concerned as much with wireworm behaviour as with the properties of the toxicants, if we wish to correctly interpret our experimental data and to efficiently develop this principle of wireworm control.

Previous to the discovery of the chlorinated hydrocarbon insecticides, most wireworm controls in field crops were cultural ones, consisting, in essence, of the manipulation of ordinary agricultural practices, or of practices which may conveniently be incorporated, to reduce wireworm damage and populations. Many cultural controls were, and still are, very useful, although the development of this approach to control has received relatively little attention in recent wireworm research, probably because cultural controls generally are more difficult to develop, and slower in effect, than the application of insecticides.

The mass of published data on the cultural control of wireworms cannot be summarized here, and it is sufficient to recapitulate that clean summerfallowing to eliminate wireworm food, crop rotation, cultivation, seeding and harvesting practices, irrigation, and soil drying, include most of the practices that have been applied, with varying degrees of success, for reducing wireworm damage and populations. Some appear only to provide protection to the crop without affecting significant population changes, while others have been developed which will reduce wireworm populations below the levels of economic importance, and maintain them there. In the Prairie Provinces cultural controls are recommended with seed treatments, or alone in fields where damage is not great enough to warrant the expense of seed treatments (McDonald and Colleagues, 1954). Clean summerfallowing every second or third year will reduce wireworm populations to levels where they will cause little damage; two or three fallows may be necessary to reduce a severe infestation as effectively as one seed treatment, but the practice will prevent an increase in populations where most of the wireworms have been killed by seed treatments. Shallow seeding in a firm, clean seed bed at the proper time, and the application of fertilizers, are recommended also to increase plant vigour and to reduce wireworm damage.

Cultural methods will always be important in the control of wireworms, but the correct approach to their development must be clearly recognized. Wireworm damage is usually influenced by a variety of environmental factors, and it is significant that each of

the most effective cultural controls that have been developed is aimed at some weak spot in the life of the wireworm where some environmental factor may be extended beyond the limits of wireworm endurance. M. C. Lane (1933), while discussing approaches to the development of wireworm cultural controls, stated that he felt "that only by intensive study of a definite wireworm species can the facts concerning its limits and endurance be determined". This approach still retains top priority in the war against wireworms, and must continue to do so, because our knowledge of the wireworms themselves will contribute more to their control than will any other factor.

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# Soil Insecticides<sup>1</sup>

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## ABSTRACT

Agricultural soils are favorable habitats for many different insects during part or all of their life cycles. Some like *Phyllophaga* and *Elatерidae* are general feeders which persist for long periods; others like *Diabrotica* are non-persistent and sometimes quite specific as to host plants.

A soil insecticide is one that is used on or in the soil to control one or more pests. Practically all of the common ones except certain systemic seed treatments are chlorinated compounds. The ones in common use are highly effective, long-lasting, economical, and non-phytotoxic at recommended dosages.

Methods of applying soil insecticides fall into three main categories, each of which has variations which are discussed. These are: (1) Broadcast treatments applied below the soil surface or promptly incorporated into it by disking, plowing, etc. (2) Liquid or dry formulations banded in or along the rows of cultivated crops. (3) Seed dressings or mechanical mixtures of seed and insecticide.

The commercial use of soil insecticides has been increasing rapidly since about 1950, particularly in the South Atlantic and West North Central States of the U.S.A. The results of treatment are often spectacular, the methods of application are convenient, and the spread between cost of and return from treatment is often high.

Crops commonly protected with soil insecticides include corn, wheat, potatoes, peanuts, turf, forage legumes, root vegetables, small fruits, sugar cane, sweet potatoes, tobacco, narcissus and nursery stock. Pests controlled include wireworms, false wireworms, root and seed maggots, rootworms, white grubs, root weevils, Japanese beetle, white-fringe beetle, flea beetles, mole crickets, ants, cutworms and sod webworms.

Soil is a favorable medium for many insects which have adapted themselves to this environment. Both food and shelter are provided and critical changes in temperature and moisture are uncommon in it. Some of our most important agricultural pests spend part or all of their lives within the soil. However, their damages are often overlooked or attributed to other causes.

Soil insects vary widely in their habits and taxonomic relationships. Coleoptera and Lepidoptera predominate, but Diptera, Orthoptera, Collembola and several other orders are represented. Some like *Phyllophaga* and *Elatерidae* persist for long periods of time and feed on a wide variety of plants. Others like the Phalaenidae are non-persistent and may not be present in numbers in a given field for more than a year or two. Other soil insects like *Diabrotica* and dipterous maggots tend to be restricted in their choice of food plants, and usually invade a field after the crop is planted.

The crops attacked by soil insects are just as variable as the pests themselves. Field crops subject to heavy damage include corn, wheat, sugar cane, forage crops and tobacco. Fruit and vegetable crops commonly damaged are potatoes, sweet potatoes, peanuts, root vegetables and small fruits. High-value crops susceptible to particular damage are nursery plantings, turf and narcissus.

## RISE OF SOIL INSECTICIDES

Early work with soil insecticides was mainly with the inorganics which were largely ineffective, and fumigants which were much too costly for most uses. It was well summarized just as the chlorinated hydrocarbon insecticides were coming into common use (Gough, 1945). The chlorinated compounds changed the whole picture from the practical standpoint, and our best soil insecticides for almost all purposes belong to this group.

The soil insecticides now in common use have the following advantages: (1) They usually are highly effective against the pests. (2) They are long-lasting so that one treatment will last for months or even years. (3) There is usually a wide margin between the recom-

<sup>1</sup>Journal Paper No. J-3079 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 1096. Accepted for publication Feb. 28, 1957.

mended dosage and the level that is detrimental to crops and beneficial soil organisms. (4) They are cheap enough to permit their use on almost any crop. (5) The results are so sure and often so spectacular that farmers readily accept them.

The first of the chlorinated compounds to be used as soil insecticides were DDT, BHC and chlordane. All three are still in use, but for many purposes they have been largely replaced by aldrin, heptachlor, dieldrin, or lindane. Endrin, isodrin and toxaphene have been used to a limited extent, but they show little promise of replacing the others in the near future. The liquid soil fumigants and the mercurials are used to some extent and certain new systemic insecticides are now coming in, but we shall not dwell on any of these in this discussion.

### FORMULATION AND APPLICATION

Insecticide formulations for soil treatment include all the common ones except aerosols. Emulsifiable concentrates, wettable powders, dusts, granulars and oil solutions all have been used in one way or another. In general if the application is properly made, a given insecticide and dosage will give equal results, regardless of the formulation employed. However insecticide placement often has a marked influence on the results obtained. For example, on a row crop like corn we recommend just double the dosage for a broadcast application that we do for a treatment banded along the rows. Also most soil insecticides are volatile, and with rare exceptions they need to be placed below the surface of the soil or intimately mixed with it in the application process or very soon thereafter.

The following methods have been used for applying insecticides to the soil. (1) Broadcast applications of sprays, dusts, granulars or insecticide-fertilizer mixtures, usually followed at once by thorough disking. (2) Use of an insecticide-fertilizer mixture in place of ordinary starter fertilizer at the time of planting row crops like corn. (3) Use of planter-mounted sprayers or dry insecticide applicators to direct the insecticide into the seed trench at the time of planting. (4) Seeding with mechanical mixtures of seed and dry insecticide (powder or granular) instead of seed alone in case of wheat, onions and a few other crops. (5) Use of insecticides in the transplant water on crops like tobacco, sweet potatoes and cabbage. (6) Dusting or dipping bulbs (narcissus) or seed pieces (sugar cane) with insecticide ahead of planting. Each method has its advantages and disadvantages for any particular pest and crop.

Putting insecticide coatings or dressings on seed is a special usage of soil insecticides which has found its widest application on corn and wheat. It has the advantages of being cheap and easy to use. Its main disadvantages are that only a very low dosage can be employed, and on row crops it is very limited in its distribution. The result is that it is at best effective only against those pests which attack the seed. Root and stem feeders are not controlled by seed treatments, and they are often the most important pests present.

### FARMER ACCEPTANCE AND USE

The real merit of an agricultural practice should be reflected in the way farmers accept and use it. Roughly  $10\frac{1}{2}$  million acres of corn are planted in Iowa each year. In 1951 we ran our first tests with soil insecticides (exclusive of seed treatments) which covered some 25 acres. During subsequent years the treated acreage has been estimated as follows: 1952—25,000; 1953—325,000; 1954—600,000; 1955—1,250,000; 1956—1,135,000. The slight decline in 1956 was due to lower farm prices and drought conditions, rather than a loss of faith in the value of soil insecticides. Each year throughout this period well over half of the total acreage was treated with insecticide-fertilizer mixtures used as starter fertilizer at the time of planting.

Two additional points need to be made regarding this development in my state. First, much of the soil-treated acreage as well as other corn was planted with insecticide-treated seed. We think of soil and seed treatment as being *complementary* rather than competitive. Second, not all of the treated acreage was known to be infested with soil insects to a damaging extent. In other words many of our farmers are sold on the idea of using soil insecticides routinely as a form of *crop insurance*. Since the cost ranged roughly from \$1.25 to \$2.50 per acre on a fairly valuable crop, the cost of this insurance was relatively low.

## WHEN ARE INSECTICIDES NEEDED?

All underground parts of plants are attacked by soil insects. Seeds, sprouts, stems, roots, bulbs and tubers are subject to damage and even total destruction. Fungi, bacteria and other soil organisms usually move in after the insects and cause secondary damage. Often the primary cause of the trouble is overlooked, or recognized too late to save the crop.

Some soil insect damages are both drastic and easily diagnosed. Most farmers and gardeners are well acquainted with cutworms and their destructiveness. Sod webworms are often associated with cutworms on sod land, and the damage symptoms are very much like cutworm injury on crops like corn. White grubs eat the smaller roots so as to cause wilting, lodging, or even death of the affected plants. Rootworm feeding on corn is the cause of a characteristic root lodging.

Sometimes even severe soil insect damage is difficult to diagnose. Loss of stand is a common symptom of subterranean insect damage on cereal grains, corn, soybeans and many vegetable crops. Wireworms, seed corn beetles, seed corn maggot and *Collembola* attack the seed so as to damage or destroy it. The result is poor and non-uniform stands of stunted, yellowish plants.

Insect populations are extremely difficult to forecast, and this is especially true of soil insect infestations. In most cases the best general criterion of what will happen in the future is the record of what has happened in the past. For example, in some parts of Iowa a farmer who plants corn following corn is sure of rootworm damage unless he uses a control measure. Corn following sod in some locations is vulnerable to cutworms and sod webworms, especially in cool, rainy seasons. A friend of mine has been on the same farm in northern Iowa for over 40 years, and experienced heavy wireworm damage in one field every time it was planted to corn until modern soil insecticides came to his rescue.

## MEASURING THE RESULTS

Actual counting of soil insects is at best a slow and tedious procedure. Samples have to be dug and carefully examined either in the field or in the laboratory. Because the populations are usually quite variable, considerable replication is necessary if convincing results are to be obtained. Soil sifting devices and bait traps are used to a limited extent, and save time and effort in specific cases, notably with wireworms.

Crop response is often the best and most convincing evidence of the value of a soil insecticide. In our work with corn rootworms we have routinely made diggings to determine rootworm numbers, rated the plants late in the season as upright or root-lodged, and measured the yields at harvest. Other criteria for evaluating the results obtained with soil insecticides on corn include plant stand, plant height, silking date and ears lost by mechanical pickers. (Bigger and Blanchard, 1955).

## WHAT ARE THE DISADVANTAGES?

A new and expanding field like soil insecticides is sure to involve some problems and pitfalls. In this instance some of these have been real and others imaginary. The real problems have been largely concerned with mixing, labeling and applying the chemicals so that the operation would be both safe and effective. Imaginary problems have arisen in the form of speculation regarding possible adverse effects of soil insecticides on crops and beneficial soil organisms.

Insecticide-fertilizer mixtures have played a key role in the advent of modern soil insecticides, and many of the real problems have been centered in the fertilizer industry. First, a uniform mixture of pesticide and fertilizer is needed. Second, it must be mixed and handled in such a way that it is safe for all who come in contact with it. Third, it must be properly labeled so that it will be sold and used as intended. Fourth, the pesticide should be mixed only with the fertilizer formulas that are also needed where the insecticide is required, and the mixtures should be used the year they are manufactured. Fifth, if they are applied broadcast they need to be thoroughly worked into the soil by disking or other means very soon after application. Generally speaking, each of these problems is being met and solved (Jacob, 1954).

The imaginary problems have pretty well solved themselves. In general the safety tolerance between the effective dosage and the level that would be phytotoxic is high.

Several published papers indicate that the recommended dosages of our most common soil insecticides (usually  $\frac{1}{4}$  to 3 pounds per acre for aldrin or heptachlor in direct soil treatments) are quite safe to beneficial soil microorganisms. The fact that these chemicals fail to control earthworms when we deliberately use them at high dosages for this purpose is satisfying evidence on this score. Likewise increased crop yields after treatment speak for themselves.

### A LOOK AT THE FUTURE

In a new field that has developed as fast as the work with soil insecticides it is hazardous to attempt a precise forecast of what is to come. In my opinion we may reasonably look for the following:

- (1) A large increase in the use of soil insecticides, both as seed treatments and in direct soil applications.
- (2) A gradual trend toward the use of granular formulations of soil insecticides applied directly in the planter trenches of row crop by the use of planter-mounted metering devices. This method apparently combines much of the economy of seed dressings with most of the effectiveness of the present methods of direct soil treatment.
- (3) The commercial use of systemic insecticides on seed to protect the young seedlings. This trend is already under way with cotton, and the method may have special merit for the small-seeded forage crops.

For anyone interested in further details regarding the present status and future possibilities of soil insecticides a more comprehensive recent review is available (Lilly, 1956).

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### DISCUSSION

R. H. BURRAGE. In seed treatment of corn with different rates of BHC in acetone, was the same amount of acetone applied in each case?

J. H. LILLY. Yes.



# The Control of Insect and Mite Pests of Fruit Trees in Northeastern United States<sup>1,2</sup>

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## ABSTRACT

Orchardists rely almost completely on chemical treatments for the control of the insect and mite pests of the tree fruits grown in northeastern United States. These forms include important pest species of both foreign and domestic origin. Because of the humid climate, fungus diseases are troublesome and require the inclusion of one or more fungicides in most of the pesticidal spray treatments applied. Apples receive approximately 12 sprays annually at a cost estimated at about \$100 per acre. Although fewer treatments are applied to peach, plum, pear and cherry, pest control constitutes a major item in their production costs. In spite of problems created by pesticide use—including human health hazards, near elimination of some natural enemies, injury and other undesirable effects on the host, and the appearance of pesticide resistant strains in pest species—fruit growers of the area are expected to continue to rely on chemical measures, primarily, for the control of insect and mite pests and of plant diseases. No other method has proved as efficient or dependable.

Measures used to control the insect and mite pests of the deciduous tree fruits differ widely between the fruit growing districts of the world. These variations occur, because of differences in the pest complex present, the climate, the horticulture practiced, the economy of the country in which the district lies, and the views of those who formulate control practices.

The cultivated tree fruits are not indigenous to northeastern United States except for some non-commercial varieties of plums. However, most of the major pests associated with these plants in Europe have now been introduced to the area. These species, along with the native insects and mites that have adopted these fruits as hosts, comprise a long and rather formidable pest list. Their collective potential for injury is probably not exceeded elsewhere in the world.

Some of the native insect species of current importance in the region, and the fruits seriously affected are: plum curculio, *Conotrachelus nenuphar* (Hbst.) (plum, cherry, apple and peach); cherry fruit flies, *Rhagoletis cingulata* (Loew) and *R. fausta* (O.S.) (cherry); apple maggot, *Rhagoletis pomonella* (Walsh) (apple and plum); red-banded leaf roller, *Argyrotaenia velutinana* (Wlk.) (apple and plum); woolly apple aphid, *Eriosoma lanigerum* (Hausm.) (apple); and the peach borers, *Sanninoidea exitiosa* (Say) and *Synanthedon pictipes* (G. & R.) (peach).

A similar list of foreign species would include: codling moth, *Carpocapsa pomonella* (L.) (apple and pear); oriental fruit moth, *Grapholitha molesta* (Busck) (peach and quince); pear psylla, *Psylla pyricola*, Foerst. (pear); European red mite, *Metatetranychus ulmi* (Koch) (apple, plum, peach and cherry); two-spotted spider mite, *Tetranychus telarius* (L.) (apple and peach); apple aphid, *Aphis pomi* DeG. (apple); rosy apple aphid, *Anuraphis roseus* Baker (apple).

Climatologically, the area under consideration lies in the humid, eastern two-fifths of the United States. The average annual precipitation in the fruit districts is around 35 inches. It ranges, between districts, from about 25 to 45 inches; the coastal areas receive more rainfall generally than do those inland. While precipitation occurs throughout the year, it is somewhat heavier during the months of the growing season, or, April through September. The climate is favorable to disease producing fungi. This situation necessitates the inclusion of fungicides in most spray treatments applied in area orchards. Insects and mites are most troublesome in the hot dry seasons; fungus diseases, in "wet" years.

<sup>1</sup> This article is based on pests of apple and on conditions prevailing in the state of New York. What is said, however, will apply in general to other fruit pests and districts of the area. For the purposes of this paper these states comprise "northeastern United States:" Maine, New Hampshire, Vermont, Massachusetts, Connecticut, Rhode Island, New York, Pennsylvania, New Jersey, Maryland, Delaware, West Virginia and Virginia.

<sup>2</sup> Journal Paper No. 1050, New York State Agricultural Experiment Station, Geneva, N.Y. October 29, 1956.

New York apple growers now apply approximately 12 pesticidal spray treatments annually to their orchards. Some limit the number to about eight; others, on occasion, may apply in excess of 20 sprays. Around 10 different pesticidal chemicals are used a season and as many as 3 or 4 of these may be combined in a single spray treatment. A typical spray program followed is given in Table I.

TABLE I—Pesticidal Spray Program Applied in an Apple Orchard at Geneva, N.Y., 1955.

Treatment name	Date applied	Pesticides used	Pest or disease control objective
Dormant	March 30	Dinitro cresol	Aphids
Green-tip	April 16	Sulfur paste & Tag**	Apple scab
Del. dormant*	April 20	Petroleum oil & bordeaux mix.	European red mite & apple scab
Pre-blossom	April 27	Sulfur paste & Tag**	Apple scab
Pre-blossom	May 2	Sulfur paste	Apple scab
Pre-blossom	May 11	Captan	Apple scab
Calyx	May 23	Lead arsenate & ferbam	Plum curculio and apple scab
10-day	May 31	Dieldrin, DDD & ferbam	Plum curculio, red-banded leaf roller and apple scab
1st cover	June 9	DDT & ferbam	Codling moth and apple scab
2nd cover	June 20	DDT & ferbam	Codling moth and apple scab
3rd cover	July 5	DDT, wettable sulfur & ferbam	Codling moth, apple maggot and apple scab
4th cover	July 18	DDT, lead arsenate & Aramite	Apple maggot, codling moth and two-spotted spider mite
5th cover	Aug. 2	DDT, DDD & Aramite	Codling moth, red-banded leaf roller and two-spotted spider mite
6th cover	Aug. 15	DDT & captan	Codling moth and apple scab

\*Delayed Dormant  
\*\*Active ingredient phenyl mercuric acetate

The annual cost of insect, mite and disease control in New York apple orchards was found to average \$98.00 per acre (von Oppenfeld *et al.* 1952). This figure was obtained from a survey made of 103 orchards in 1949 and 116 in 1950. In these studies, 63% of the cost was for spray materials (2/3 of this for insecticides and acaricides), 10% for labor, 7% for tractor power and 20% for spray equipment.

Although fewer spray treatments are applied to the other tree fruits, pest control constitutes a major item in the production costs of all of these fruits. As with apple, most treatments applied contain both a fungicide and insecticide. In New York, growers apply approximately 7 pesticidal sprays to cherry, 8 to peach, 6 to plum and prune and 6 to pear.

GENERAL DISCUSSION

Since pesticide usage on orchard crops in the Northeast is probably not exceeded elsewhere in the world, some readers may question both the entomological and economic soundness of these practices. The writer will attempt to explain why these practices are being followed through a discussion of the four questions which follow:

1. What degree of control can be expected from biological agents?
2. Assuming the necessity of using some chemical treatments, to what extent is it practicable to harmonize chemical and biological control?
3. It is really necessary to apply as many spray treatments as growers of the area now use?
4. Ultimately, will not such problems as the development of pesticide resistant strains, human health hazards, excessive accumulation of pesticides in the soil, etc., force less reliance on chemical control?

POSSIBILITIES OF BIOLOGICAL CONTROL

Fruit pests of the Northeast are subject to attack by normal numbers of parasitic and predaceous arthropod species. However, no economic entomologist would suggest that fruit

growers could obtain desired levels of pest control through reliance on biological agents alone. That this is generally true in the case of intensively grown crops is pointed out by Clausen (1956): "only a small portion of our more important agricultural pests can be fully controlled by this method and that with a substantial number of them, no reduction whatever can be attained."

Natural enemies are particularly ineffective in controlling the important internal fruit feeders, notably, codling moth, apple maggot, plum curculio and the cherry fruit flies. Ample evidence to support this statement will be found in the abandoned plantings of the area. As noted by Clancy and McAlister (1956), parasites and predators are most effective against species which are more or less continuously exposed to attack, as with aphids, scale insects, mites and surface feeding caterpillars.

An indication of the local severity of perhaps the most important of the internal feeders—the codling moth— is given in Table II<sup>3</sup>. The orchard described is one maintained for the primary purpose of evaluating insecticides for codling moth control (Glass, 1954). Approximately half of the trees in this block, or less, are used annually for insecticide tests; the remainder receive fungicidal sprays only, with a few exceptions, because of the necessity of keeping apple scab under control. While obviously natural enemies are not permitted to function at full efficiency in the insecticidally-untreated trees, both because of the presence here of fungicides and their nearness to trees bearing insecticides, the records do indicate that the codling moth is far too destructive locally for growers to place much reliance on biological agents.

TABLE II—Codling Moth Infestation in Insecticidally-untreated Apple Trees and those Receiving DDT. Geneva, N.Y.

Year	Ave. temp. °F. May, June, July, August	No. DDT Cover Sprays	Per cent wormy 1st brood (untreated)	Worms/100 apples at harvest	
				Untreated*	DDT Treated**
1948	66.0	5	31	130	2
1949	69.3	5	53	202	25
1950	65.3	5	28	145	1
1951	65.5	5	44	206	2
1952	66.2	5	18	139	7
1953	66.5	5	50	297	2
1954	65.3	5	37	244	1
1955	69.5	6	54	211	5
1956	63.8	5	11	118	1

\*Trees received fungicide sprays but with a few exceptions no insecticides or acaricides. No consistent fungicidal program has been used over the years; it was modified from year to year on the advice of Station Plant Pathologists.

\*\*Used at rate of 1 lb. of actual DDT in wettable powder formulations in 100 gallons of water.

A more successful adaptation of a native pest species to a new situation than of its parasites appears to have accounted for reduced parasitism in at least one instance. Reference is made to the apple maggot and its parasites of the genus *Opius* (Porter, 1928). The ovipositor in these species is of sufficient length to reach the larva of *R. pomonella* when it occurs in the small diameter fruits of its original hosts, presumed to be haws (*Crataegus* spp.) and possibly also, blueberries (*Vaccinium* spp.) and huckleberries (*Gaylussacia* spp.). This is not the case where the much larger fruit of the apple is involved. Thus *Opius* parasitism in apple maggot developing in apple fruits ranges from a trace up to a maximum of only 3–4 per cent in the writer's experience<sup>4</sup>; parasitism may reach nearly 50 per cent where the blueberry maggot is involved (Lathrop and Newton, 1933).

While modern pesticides may be highly toxic to entomophagous forms, it is not clear to what extent and in what way their use was a factor in bringing about the changes that have been noted recently in the area orchard pest fauna. Thus since DDT was intro-

<sup>3</sup> Investigators of the codling moth will recognize that any insecticide evaluated under conditions such as these is put to severe test. Moths developing in the insecticidally-untreated trees will give rise to a much larger second brood throughout the orchard than would have been the case had efficient orchard-wide control been practiced. Relatively, therefore, DDT has given consistently good control here. In commercial orchards, DDT enables local growers to produce essentially "wormfree" crops.

<sup>4</sup> Unpublished records.

duced mites have become much more active and destructive; and, insects such as the red-banded leaf roller which previously had been of minor importance suddenly became major pests (Glass and Chapman, 1952; Clancy and McAlister, 1956). Not all of these changes can be attributed to drastic reductions in the numbers of natural enemies by pesticides but this must have been one of the major contributing factors.

A considerable amount of biological control activity occurs in area orchards in spite of widespread use of toxic chemicals. It may, in fact, be on the increase. Thus, aphid populations attract goodly numbers of syrphids, coccinellids and chrysopids and the coccinellid *Stethorus punctum* (Lec.), an active local predator of phytophagous mites, has become increasingly common within recent years. It appears to have developed some resistance to DDT. Even the *Typhlodromus* mites are often common in fully sprayed plantings. The foregoing subject has been reviewed recently by Ripper (1956).

Fruit growers of northeastern United States will doubtless continue to make extensive use of pesticidal chemicals even if in so doing they may create problems by upsetting favorable biological balances in their orchards. Interest exists, however, in learning what can be done to harmonize biological and chemical control. This brings us to the second question listed.

#### HARMONIZING BIOLOGICAL AND CHEMICAL CONTROL

This concept entails the use of pesticides which are so selective in their toxicity as to control pest species but will spare their more important natural enemies. A. D. Pickett and his associates (Pickett and Patterson, 1952) and Clancy and McAlister (1956) have explored the possibilities of this method rather extensively under Nova Scotia and West Virginia conditions, respectively. A considerable degree of this desired harmony is apparently being achieved in spray programs currently being used by growers in Nova Scotia. LeRoux (Ripper, 1956) has also demonstrated the applicability of the principle in Quebec. Marshall and Morgan (1956) conclude, however, that primary reliance cannot be placed on natural enemies to control apple pests in western Canada, principally because of the local severity of pests like the codling moth. However, these workers do favor limiting the amount of spraying done, using corrective more than preventive measures.

Any attempts made to adjust chemical control programs to protect parasites and predators will need to reconcile these objectives with those for plant disease control. For, as previously noted, most spray treatments applied in the area contain both fungicides and insecticides (Table I). This situation may create additional complications. Some fungicides, for example, are at least moderately toxic to both phytophagous and entomophagous arthropods (Clancy and McAlister, 1956; Lathrop, 1955; Lienk and Chapman, 1952; and Pickett and Patterson, 1952). Sulfur is cited as being particularly objectionable in connection with the latter group.

The American chemical industry exerts a potent effect on pest control practices. Thus, its dynamic and highly competitive character results in the introduction of a steady stream of new pesticides and the ushering out of older materials as they become outmoded. This is a desirable, even necessary, process where pest control is based primarily on the use of chemical treatments. It does create, however, a rapidly changing scene and one that allows far too little time to work out, for the pesticide complex in vogue at a given time, an intelligently based adjustment between chemical and biological control.

At present little conscious effort is made to harmonize chemical and biological control in orchards of northeastern United States. Economic considerations require that crops be produced which are essentially free of all insect and disease damage or infestation. At present this goal can only be attained through the extensive and intensive use of pesticides.

#### NUMBER OF TREATMENTS NEEDED

Probably no successful fruit grower anywhere applies more pesticidal spray treatments than he believes are necessary to insure the production of a readily saleable crop. What this "necessary" number is, however, will vary depending primarily on the market standards the crop must meet. If to some readers this number still seems excessive in northeastern United States, possibly a review of the forces operating here may show why this course is being followed.

Basically these forces are economic: the competition that exists between the same and other fruits in the market place; purity and quality standards established by official agencies; and, similar standards of food processing companies.



Fresh fruits are sold in American markets largely on the basis of eye appeal. Area growers find it necessary to offer an attractively colored, blemish-free product and of course one that is free of internal or external insect infestation. Products not meeting these standards simply lose out to those that do. To attain the foregoing objectives a successful grower early learns he can leave little to chance in his spraying operations. Some treatments will be applied as insurance measures. This practice will more often apply to fungus disease control than to that for insects and mites; however, it may be true for the latter purposes too.

What took place in regard to the control of the oriental fruit moth in peach in New York is a good illustration of the relation of consumer acceptance of a product to pest control practices. Prior to the introduction of DDT, reliance was placed on the control of this pest by natural enemies, notably by *Macrocentrus ancylivorus* Roh. However, the occurrence of 10 to 15% "wormy" peaches on the average under this program was accepted by the housewife and food processor only because of the absence then of a more effective control. With the introduction of DDT a grower was offered this choice: continue to rely on parasites or, add to production cost the new expense of an insecticidal spray program. There was little hesitation evidenced as to which method was preferred. The grower elected to use DDT. (Organophosphorous materials have now largely replaced DDT for this purpose). The spraying produced peaches almost completely free of oriental fruit moth infestation and thereby made the fruit much more acceptable than it previously had been under the natural control program.

Another example is offered by the pie or sour cherry crop. Most of this fruit in the area is frozen or canned. State and federal pure food officials will not tolerate any degree of insect infestation in the marketed product. Infestation here consists of the larvae of the plum curculio and of the two trypetids, *Rhagoletis cingulata* (Loew) and *R. fausta* (O. S.). To meet the standards established, cherry growers are obliged to spray thoroughly and often. The final insecticidal spray, for example, may be applied only a day or two before fruit harvest starts. (Fortunately, lead arsenate, the insecticide commonly used, is readily removed in the pre-packing soaking and washing operations to which the fruit is subjected.)

The situation relative to the apple maggot and the food processing companies offers yet another example of the influence of consumer acceptance on pest control practices. More than half of the apple crop of New York is processed into sauce, slices, juice, etc. Companies purchasing apples for the first two purposes will not accept fruit containing any internal insect infestation, or discoloration or streaking left by an insect puncture or burrowing larva. Apple maggot is the common local offender in connection with the foregoing type of infestation. To meet the requirements of food processors on apple maggot, some growers are obliged to do more spraying than might otherwise be the case.

There has been a considerable increase in the amount of spraying done in area orchards over the past two decades. Curiously, part of this increase can be attributed to rising labor costs. One such effect was to stimulate the development of spraying equipment that could be operated with a minimum of man power. The air blast sprayer, so widely used today, is a direct result of this effort. With such equipment, one man can now treat upwards of 100 acres a day—a job formerly requiring possibly 3 or 4 men and a like number of days. Availability of such equipment has reduced the per acre costs of individual spray treatments and removed much of the former drudgery associated with the operation. Because spraying has been made easier, in a sense, area growers take fewer chances; in cases of doubt, they are more inclined to apply the spray than to withhold it.

Also related to high labor costs is the view held by many local growers that it is more economical to obtain near perfect insect and disease control by spraying intensively, rather than accept, under a limited or less efficient spraying program, an appreciable percentage of injured fruit which will have to be sorted out at harvest. Aside from the added labor costs for grading, there is the loss of the culled fruit, which either must be sold at a lower grade or discarded. Under the former plan, growers may not need to do any appreciable harvest grading. The fruit either is transferred directly from the picking bag or pail to the container in which it is sold—if the crop is destined for processing—or held in similar containers for packing out later as fresh fruit.

#### PROBLEMS CREATED BY PESTICIDE USE

Almost everyone is aware there are disadvantages in using toxic chemicals to control crop pests. Rather than being unique, this situation is common to all human endeavor.

We elect to use practices where the advantages in so doing appreciably outweigh the disadvantages, and especially so, if no satisfactory alternative course is available. Thus, the occurrence of problems in pesticide use is not a particularly valid argument *per se*, against the practice. A discussion of several of the more serious pesticide-created problems follows.

The ability of pest species to develop pesticide resistant races is presenting an increasingly serious problem along many fronts. Among fruit pests in the Northeast, it has complicated the control of three species to date, *viz.* codling moth, European red mite and red-banded leaf roller.

Pesticide resistance is not a new problem with the codling moth. Thus, lead arsenate resistant strains of the species have occurred in many area plantings for more than 25 years. Hough (1943), in his classical studies on this subject, found "resistance" not only included a tolerance to lead arsenate but also to nicotine and fluorine compounds. Recently, the beginnings of DDT resistance were detected in a few orchards in western New York (Glass and Fiori, 1955). It seems likely growers, ultimately, may need to abandon DDT for codling moth control. To date, however, almost all area growers continue to rely on DDT and lead arsenate for the control of this species.

The occurrence of European red mite resistance to parathion and some other organophosphorous pesticides was first established in 1952 (Lienk *et al.*, 1952). It is now generally appreciated that any sustained use of these products is apt to result in the development of phosphate-resistant strains. In New York, only plum and peach receive such pesticidal programs. Actually "phosphate-resistance" has not created a real problem for growers to date since petroleum oil and other good non-phosphate acaricides are available.

The red-banded leaf roller is the newest fruit species of the region to develop pesticide resistance and in this case to DDD. Suspected in the fall of 1955, its occurrence was definitely established in 1956 (Glass, 1957). This development is too current to assess its seriousness or to say which of several possible insecticides can be substituted for DDD.

Summing up the present status of pesticide resistance: It is recognized as a problem and potentially a very serious one; instances of its occurrence in orchards of the Northeast are limited as yet to a relatively few plantings and species; and thus far effective alternative pesticides have been available for grower use where it has occurred.

Great strides have been made in the United States within recent years to provide adequate health safeguards for those who manufacture pesticides, apply them to control pests, and to consumers who eat foods that were treated with these chemicals in their production. Dangers appear to have been reduced now to manageable proportions in all theatres of possible exposure. The Pesticide Amendment to the Food, Drug and Cosmetic Act (Miller Bill) which became law in 1954 seems to have provided an orderly and workable procedure for meeting the requirements of all concerned with the problem. First, it endeavors to provide full protection of the public health. However, it does recognize that farmers need to use pesticides to produce their crops; and that the dynamics of pest control with chemicals cannot be served adequately unless reasonable provisions are made for the introduction of new materials.

Probably pesticide use will always entail human health hazards. But experience to date suggests that except for accidents and deliberate misuse, pesticides can be employed safely.

And then there are those actual and potential problems involving the undesirable effects of pesticides on the host plant under treatment. These range from visible burning or other injury to the leaves or fruit to subtle indirect changes in the color or flavor of products manufactured from the fruits. Most such difficulties are encountered during the initial stages in the use of the pesticide. If such shortcomings prove uncorrectable the material may need to be abandoned outright. Often, however, such deficiencies can be surmounted by one of these means: by improving the manufacturing process to eliminate an offending impurity, or by refinement of the basic chemical itself; by altering the formulation; by reducing the dosage used on the plant at one time; or by avoiding incompatible combinations with other pesticides.

Concern has also been expressed over the likelihood of poisoning the trees, ultimately, from excessive accumulations of pesticides in the soil from spray run-off. A situation approaching this condition occurred in orchards of the Pacific Northwest during the late twenties and thirties when lead arsenate was used so extensively for the control of the

codling moth. While deep rooted tree fruits apparently were not harmed by these soil accumulations, it became increasingly difficult to maintain or establish cover crops in these orchards. Furthermore, vegetables and other crops planted on the land directly after the orchards had been removed were affected adversely (Vincent, 1939). Ginsburg and Reed (1954) have published some interesting data showing DDT levels in the soil of typical New Jersey orchards. While the occurrence of such residues in orchard soils will bear close watching for signs of tree distress or other harmful effect, the matter appears to be more of a potential than actual problem at present.

### CONCLUSIONS

The foregoing account has been presented in the nature of an interpretative report. Thus the writer has endeavored to describe the pest control practices area orchardists employ and why they employ them. What follows now will more directly represent the writer's views and it will also include a look to the future.

First, we should recognize that almost everything we do in an orchard including the orchard itself is "unnatural".

Consider these facts: We group large numbers of the same kinds of trees together into "orchards" and thereby provide pests with an almost unlimited supply of food in one place; we insist on growing varieties that are highly susceptible to pests and diseases (the McIntosh apple for example); we prune and feed the trees to produce maximum and annual fruiting; we cultivate the orchard soil or plant a specific ground cover and mow it periodically; in some areas additional water is provided as irrigation; we bring in colonies of bees for pollination, or we hand pollinate; we apply growth regulating chemicals, early, to thin the crop, and at harvest to prevent fruit dropping; we store the crop under refrigeration and often in addition under controlled atmosphere conditions. Finally, it is true, we use chemical treatments to control pests and diseases. But is this last named practice essentially any more unnatural than the others?

The future importance of biological agents in the control of orchard pests in northeastern United States is not clear. It might well be a stronger force than it is today. A more definite place, for one thing, may be found for microbial organisms. The possible use of the granulosis disease to control the red-banded leaf roller is a specific example (Glass, 1958). Greater participation of entomophagous species in control may be realized, either through their development of strains resistant to pesticides in use, or through the adoption or selection of pesticides which are tolerated by key natural enemies, or in both ways. That the former possibility may well be realized is suggested by the studies of Pielou and Glasser (1952). They were able to develop a DDT-resistant strain of *Macrocentrus ancylivorus* Roh., an effective parasite of the oriental fruit moth. Reference has already been made to similar developments, apparently under orchard conditions, in the case of predaceous mites that attack phytophagous mite species.

In view of the likelihood that entomophagous species are as capable of developing pesticide resistant strains as are phytophagous forms, one is lead to wonder if a fruit grower's interests would not be better served by using a pesticidal program that will give maximum pest control, rather than run the risk of excessive crop losses by attempting to protect the parasite and predator through use of an abbreviated spray program involving so-called natural enemy-tolerant chemicals.

Questions like the one just posed cannot be resolved except through future experience and research. We need more research that bears directly on this important question. That reported by Pickett and Patterson (1953) and Clancy and McAlister (1956) are good examples of what is needed.

Falling into a similar category are efforts designed to measure the injury-producing capacity of those pests which do not attack the fruit directly. The mite injury studies made at this Station are examples of the kind of effort referred to (Chapman *et al.*, 1952; Lienk *et al.*, 1956; Boulanger, 1958). Such studies should determine what population level must be attained before the quantity or quality of the crop begins to be affected adversely. For obviously, measures should not be applied to control these species except to avert economic damage.

While the advances that have been made in the pesticide field have been phenomenal, especially within the past decade, there is ample room for improvement. Short range im-

provements may well arise from the development of more and better examples of effective pesticides having low mammalian toxicity; of products possessing a similar toxicity differential between phytophagous and key entomophagous forms to the advantage of the latter; of "resistance-proof" materials, that is, chemicals which will not be rendered useless, practically, by the development of highly resistant strains; and of products giving prolonged protection against pests or plant diseases—possibly by systemic action—and thereby reducing the frequency of treatment.

Regardless of what the future may hold, the writer has every confidence in the ability of the entomological profession to continue to provide fruit growers with effective means of controlling pests. Undoubtedly better control measures can be evolved—safer, less expensive, simpler, and less laborious. These improvements will be made as our knowledge increases and especially if research is pressed on the broadest possible front.

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# The Effect on the Balance of Arthropod Populations in Orchards Arising from the Unrestricted Use of Chemicals

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## ABSTRACT

*A great variety of harmful phytophagous Arthropods were associated with deciduous fruits in England during the first twenty years of the present century. Since the introduction of chemicals into orchard practice, however, the picture has changed considerably; today far fewer pest species occur, but even so the damage caused by the few remaining may be as serious as formerly.*

*The effect on the fauna of the application of chemicals, which have been in general use for the past thirty-five years, may be very considerable. Not only do certain species vary in numbers from season to season, but their importance as pest species changes also; for example harmful species build up and become increasingly important, while once familiar pest species disappear or become relatively unimportant.*

*In addition to the pest species, extensive surveys made in both derelict and sprayed orchards show that over one hundred species of insects, mites and spiders known to be partly predacious in habit are also present. The presence or absence of these beneficial species is closely related to the spray programme; for example, in orchards where DDT, BHC or phosphorus insecticides are applied there may be a marked reduction or complete absence of beneficial insects until June, and where DDT is used to control Codling Moth they may be absent throughout the summer. In derelict orchards, however, there is a succession of beneficial species throughout the year.*

*Future research should be directed to explore the practicability of maintaining a succession of a few of the more important beneficial species in the orchard throughout the season. This might be achieved by modifying the existing spray programme by the use of selective chemicals and the adjustment of the timings of spray applications, thereby harmonizing the chemical approach and biological control.*

Chemicals have been used to control phytophagous arthropod infestations of deciduous fruit, for many years in Europe, and for all but a few minor pest outbreaks, chemical control is the only practical method so far available.

It has been the practice of those economic entomologists interested in fruit pests in the British Isles, to make detailed studies of the life-cycle and habits of the more important fruit pests in order to discover the critical stage of their cycle at which control measures can best be directed against them. Then follows a number of carefully planned field spraying trials, with a wide range of chemicals of differing formulations and strengths, with a view to the control of the pest. Such investigations are invariably carried out against specific pests without any reference to the numerous other arthropods present in the orchard, and even without regard to the rest of the spray programme being followed in the orchard.

This haphazard and unscientific approach to the problem, without regard to the overall effect of the sprays on the orchard fauna, frequently results in substantial population changes and in the introduction of new pests.

Before considering these population changes in greater detail, and citing well-known examples, it is expedient to discuss in some detail the arthropods associated with unsprayed or derelict orchards.

The fauna of the unsprayed orchard is extremely varied and covers a very wide field of the Insecta. For example, no less than nine orders of the Insecta are represented: Dermaptera, Psocoptera, Thysanoptera, Hemiptera-Heteroptera, Hemiptera-Homoptera, Neuroptera, Lepidoptera, Coleoptera, Hymenoptera and Diptera. Although the survey of the insects associated with unsprayed orchards is by no means complete, already well over one hundred species are recognized on tree fruits alone. In addition to the Insecta, the Orders Acarina and Arachnida contain important members of the fauna.

The kinds of insects associated with tree fruits may for convenience be divided into three groups, namely, the injurious phytophagous species, the true parasites and predatory species, and those best described as neutral species.

It will not be expedient to remark upon all the species associated with the unsprayed orchard, but reference will be made to some of the more important harmful and beneficial species.

### HARMFUL SPECIES FOUND IN DERELICT ORCHARDS

DERMAPTERA: *Forficula auricularia* L.

THYSANOPTERA: *Thrips angusticeps* Uzel.

HEMIPTERA-HETEROPTERA: *Plesiocoris rugicollis* (Fall.), *Lygus pabulinus* (L.).

HEMIPTERA-HOMOPTERA: *Rhopalosiphum insertum* (Wlk.), *Aphis pomi* (DeG.), *Sappaphis mali* Ferr., *S. devector* (Wlk.), *Eriosoma lanigerum* (Haus.), *Psylla mali* Schm., *Lepidosaphes ulmi* (L.), *Eulecanium coryli* News., *Aspidiotus ostreaeformis* Curt., *Typhlocyba froggatti* Baker, *T. rosae* (L.), *Cercopis vulnerata* Ger.

LEPIDOPTERA: Geometrid species including *Operophtera brumata* (L.), *Alsophila aescularia* Schiff., and *Erannis defoliaria* (Clerck); Tortricid species including *Cacoecia oporana* (L.), *Ditula angustiorana* Haw., *Spilonota ocellana* (Schiff.), *Pandemis heparana* (Schiff.), and *Ernarmonia pomonella* (L.); *Yponomeuta malinella* (Zell.), *Argyresthia conjugella* Zell., *Blastodacna atra* (Haw.), *Lyonetia clerkella* (L.), *Coleophora nigricella* (Step.), *Anthophila pariana* (Clerck).

COLEOPTERA: *Melolontha melolontha* (L.), *Otiorrhynchus singularis* (L.), *Phyllobius oblongus* (L.), *Anthonomus pomorum* (L.), *Scolytus rugulosus* (Ratz.).

HYMENOPTERA: *Hoplocampa testudinea* (Klug).

DIPTERA: *Thomasiniana oculiperda* (Rueb.).

ACARINA: *Metatetranychus ulmi* (Koch), *Tetranychus telarius* (L.), *Bryobia praetiosa* (Koch).

About one hundred species of harmful insects and mites occur upon unsprayed apple trees in Britain, and amongst these the following are regarded as being some of the most important. The Apple Sucker, the Scale insects, the caterpillars of the Winter Moth group, the Apple aphids including the Woolly Aphid, and the Codling Moth. It is significant that the Bryobia Mite is invariably present in unsprayed orchards and frequently causes considerable damage, whilst the Fruit Tree Red Spider Mite and the Red Spider Mite although present in very small numbers never build up sufficiently to cause any harm. It is also interesting to note that both the Apple Capsid Bug and the Common Green Capsid, both once regarded as very important fruit insects, rarely occur commonly in unsprayed orchards.

### PREDACIOUS SPECIES FOUND IN DERELICT ORCHARDS

THYSANOPTERA: *Aeolothrips melaleucus* Hal.

HEMIPTERA-HETEROPTERA: *Anthocoris confusus* Reut., *A. nemoralis* (Fab.), *A. gal-larum-ulmi* (DeG.), *A. nemorum* (L.), *Orius niger* (Wolff), *O. majusculus* (Reut.), *O. minutus* (L.), *O. laevigatus* (Fieb.), *Phytocoris tiliae* (Fab.), *P. reuteri* (Saund.), *P. ulmi* (L.), *Camptobrochis lutescens* (Sch.), *Deraeocoris ruber* (L.), *Campyloneura virgula* (H.S.), *Pilophorus perplexus* D. & S., *Blepharidopterus angulatus* (Fall.), *Orthotylus marginalis* Reut., *O. ochrotrichus* Fieb., *Capsus meriopterus* (Scop.), *Malacoris chlorizans* (Pan.), *Psallus ambiguus* (Fall.), *P. variabilis* (Fall.), *Atractotomus mali* (M.D.), *Plagiognathus arbustorum* (Fab.), *Campylomma verbasci* (M.D.).

NEUROPTERA: *Eumicromus angulatus* Steph., *Chrysopa carnea* Steph., *Conwentzia psociformis* Curt.

LEPIDOPTERA: *Cosmia trapezina* (L.).

COLEOPTERA: *Oligota flavicornis* Bois., *Tachyporus hypnorum* (Fab.), *Adalia decempunctata* (L.), *A. bipunctata* (L.), *Coccinella septempunctata* (L.), *Exomias quadripunctulatus* (L.), *Stethorus punctillum* Weise.

DIPTERA: *Feltiella tetranychii* Reub., *Arthrocnodax wissmanni* Kief., *Syrphus ribesii* (L.), *Scaeva pyrastris* (L.).

ACARINA: *Allothrombium fuliginosus* Herm., *Anystis agilis* Banks, *Typhlodromus tiliae* Oud., *T. finlandicus* Oud., *T. masseei* Nes., *Phytoseius spoofi* Oud.

ARACHNIDA: Of the true spiders only species contained in the families Theridiidae and Linyphiidae, and in particular *Theridion pallens* Bl., have been recorded.

Of the predacious species of insects, mites and spiders associated with the phytophagous fauna of the unsprayed apple orchard, no less than twenty-five species of Hemiptera-Heteroptera have been recorded. These species belong to the families Anthocoridae and Miridae which contain some of the most important beneficial species associated with fruit trees. They are the Black-kneed Capsid, *Blepharidopterus angulatus* (Fall.); *Psallus ambiguus* (Fall.) and *Anthocoris nemorum* (L.). These three species are predacious upon the Fruit Tree Red Spider Mite, and occur in both commercial and unsprayed orchards, but are less common in the former.

The Order Coleoptera is represented by a number of important species, including the Two and Seven Spot Ladybird Beetles, and the minute black species *Stethorus punctillum* Weise. Their diet is varied but all three feed upon the Fruit Tree Red Spider Mite and the apple aphids.

The most important *Acarus* in the unsprayed orchard is *Typhlodromus finlandicus* Oud. which feeds upon the Fruit Tree Red Spider Mite and the species of Phytoid mites found on apple.

To-day over one hundred predacious species of insects, mites and true spiders are found in derelict orchards in about equal numbers, and most of them have been observed to prey upon the phytophagous fruit mites. It should be noted also that there is a succession of these species in the orchard throughout the year, feeding upon the immature and adult forms of the phytophagous mite in the spring and summer months, and upon the winter eggs during the dormant season. This continuous predation doubtless accounts for the scarcity of the Fruit Tree Red Spider Mite in derelict orchards.

Before spraying became a general practice from 1921 onwards the fauna of both commercial and derelict orchards was very similar, and indeed most of the harmful and beneficial species mentioned earlier in this discussion occurred equally commonly in both sprayed and unsprayed orchards. This is not surprising since the application of spray chemicals was very limited, and pruning operations and general orchard hygiene were only conducted on a small scale. The progressive growers of those days largely relied upon ducks, geese and poultry to control their pests, and grease-banding the trees was the fashion of the day. None of these practices was successful in reducing pest infestations to any marked extent. The first real change of the faunal populations of commercial orchards compared with those of derelict ones occurred in 1922 when the hydrocarbon oil or tar oil winter wash was introduced into the spray programme. The marked effectiveness of the winter wash in eradicating a number of very important pests at once became apparent to the grower, and the winter wash was applied to his trees each winter as a routine spray. Amongst the important pests controlled were the Mussel Scale, the Nut Scale and the Oystershell Scale. Complete control of the Rosy Apple Aphid, the Rosy Leaf-curling Aphid, the Green Apple Aphid, Apple Sucker, and a partial control of the caterpillars of the Winter Moth group and of the Woolly Aphid was obtained.

The tar oil winter wash also killed the moss, lichen and algae growing on the trees, and caused the rough, loose bark to peel off the trunks and main branches. In effect this removed the hibernating quarters of many insects, and those which were not killed by the winter wash sought alternative shelter for the winter months. A number of beneficial insects were known to hibernate on the trees, and amongst these the Anthocorid bugs were either killed or moved elsewhere. Indeed the absence in spring months of the various species of Anthocorid bugs from orchards which received a winter wash was noteworthy. In some seasons the summer generation of the Anthocorid bugs returned to the orchards from adjacent hedgerows and woods during the late summer and autumn, only to be killed by the winter wash which was applied later in the year.

A few years after the introduction of the winter washes, very substantial increases of the Fruit Tree Red Spider Mite were noted in orchards where the winter wash had been

applied regularly each winter. In fact the mite became so plentiful in British orchards that it has come to be regarded as a major pest of apple and plum orchards in the South-Eastern and Eastern Counties.

Many hypotheses have been advanced to account for the rapid rise of mite populations following the application of the tar oil washes, but so far the complete story has not been told. It was soon established that the tar oil washes were not toxic to the winter eggs of the mite; and as already stated, these washes provided a general 'clean-up' of the trees, destroyed many beneficial insects and removed the hibernating quarters of others. Amongst the beneficial species affected were the Anthocorid bugs, the Lady-bird beetles, and minute predacious mites, all of which are known to feed upon the phytophagous species during the spring and summer months. It is suggested, therefore, that the destruction of the beneficial species by the winter wash, and the departure of others because of the removal of their winter quarters, may partly account for the build-up of the harmful species during the spring months.

Another very striking example of an Arthropod changing its habits and eventually becoming a serious apple pest is provided by the Mirid bug, *Plesiocoris rugicollis* (Fall.), popularly known as the Apple Capsid Bug. The Apple Capsid Bug first became established in a few commercial apple orchards in Cambridgeshire following the first World War, and in a few years it had established itself in most fruit growing districts of this country, the notable exceptions being the greater part of Essex and the Tenterden district of Kent. The absence of the bug from these areas has not been explained. By 1931 the Apple Capsid Bug was so prevalent in commercial orchards that the grower rightly regarded it as one of the most serious apple pests.

It is noteworthy that the spread of the Apple Capsid Bug in commercial orchards coincided with the modification of the spray programme, and the possible correlation between the two is even more suggestive when one recalls that the Apple Capsid Bug is never common in unsprayed orchards, if indeed it is present at all.

The destructive nature of the Apple Capsid Bug and the Fruit Tree Red Spider Mite led to a further modification of the spray programme by the introduction of the petroleum oil and the DNC-petroleum wash. In British orchards the DNC is usually applied at the delayed-dormancy period, in admixture with petroleum oil. The petroleum oil is used against the winter eggs of the Fruit Tree Red Spider Mite and the Apple Capsid Bug, and the toxicity of DNC to aphids. In spite of the new delayed dormancy ovicides, however, the problem of adequate mite and capsid control became more and more urgent.

The DNC-petroleum delayed dormancy wash, which largely superseded the tar oil wash, also caused an almost complete elimination of the few important beneficial species which still hibernated on the tree, the important exception being the Black-Kneed Capsid which survived not only the tar oil wash but also the delayed dormancy washes in general use.

More pronounced changes of the orchard were observed in 1946, 1947 and 1948 following the introduction of the chlorinated hydrocarbon insecticides. Both DDT and BHC preparations were soon available in several formulations, and both proved very effective synthetic insecticides against a number of important fruit insects still infesting apple orchards, such as the Apple Capsid, Apple Blossom Weevil, Apple Sawfly, caterpillars of the Winter Moth group, Tortricid caterpillars, Codling Moth and the Apple Aphids.

It has been found that both DDT and BHC are toxic to most of the beneficial insects and mites which occur on apple, thus when these chemicals are used after the blossom period in orchards where red spider is serious, substantial increases of mite populations are likely to occur. It has therefore been suggested that their use be restricted to early pre-blossom periods only. In practice many growers apply BHC at petal-fall to control apple Sawfly and DDT in late June to control Codling Moth. The latter of course results in the complete elimination of the beneficial insects inhabiting the trees at the time of application.

All this time serious outbreaks of the mite were still causing anxiety to growers in Kent and the Eastern Counties. This led to a further modification of the spray programme by the introduction of the organophosphorus chemical parathion. Parathion was applied



twice at the fruitlet stage and immediately caused a sharp decline of the mite populations during the early summer months, but later in the season the populations built up again very substantially, culminating in higher infestations than ever before. In consequence some growers in the Eastern Counties applied four or even five applications of parathion post-blossom in an attempt to stamp out the mite. This unwise procedure was not successful, however, since even greater mite populations built up on the trees, and in addition a second species of mite, namely, the Red Spider Mite (*Tetranychus telarius* (L.)) also occurred in pest proportions and caused much injury to the trees. No predacious insects or mites were found in the orchards which had been sprayed excessively with parathion, and their absence may account in part for the abnormal increases of the two species of phytophagous mites.

In 1953 the spray programme was modified again by the introduction of the summer ovicides chlorbenzide and chlorfenson. The expression 'summer ovicide' is somewhat unfortunate since the chemicals are effective against the immature stages, and to a lesser degree the adults, as well as the ova of the mite. These new ovicides are recommended for use at various times of the season, first at the pink bud stage against the winter eggs of the mite; second, at petal-fall to kill the newly hatched mites; and third, at the fruitlet stage against the summer eggs and immature stages. Under British conditions the fruitlet sprays have given the best results.

These summer ovicides have provided a good commercial control of the mite, but even so in most orchards the population tends to build up again in the autumn months to a high level, making further applications necessary the following summer. The evidence so far available suggests that the summer ovicides are not toxic to the beneficial insects associated with apple. The acceptance of the summer ovicides into the spray programme is a step in the right direction since they replace the organophosphorus chemicals which proved so harmful to beneficial species.

It will be noted that the modification of the spray programme has been going on every few seasons over the past thirty-five years, and even today new pesticides are being introduced into the spray programme. During this period a number of species once familiar in the commercial orchard have disappeared, but even so, those that remain often reach pest proportions in some seasons. For example, such pests as the Oat Apple Aphid, the Green Apple Aphid, the Woolly Aphid, the Apple Sawfly, the Codling Moth, the Summer Fruit Tortricid, the Fruit Surface-eating Tortricid and the Fruit Tree Red Spider Mite are usually present in most commercial orchards and many build up rapidly when conditions allow.

In addition to the steady stream of new chemicals, and the many modifications of the spray programmes every few years, the improvements and the modifications of the spraying equipment and machinery are no less striking. For example, the low volume spraying machines are beginning to replace the once familiar hydraulic high volume machines. These low volume machines are becoming very popular because they conserve labour, cover a greater acreage of fruit per day and apply considerably less diluted spray per acre.

In practice the same amount of spray concentrate is used in the low volume machines as with the high volume, but the concentrate is added to a smaller quantity of water. In fact the low volume machines available apply as little wash as fifty, thirty or ten gallons per acre. In practice if the spray is to be applied at fifty gallons per acre the concentrate is used at five times the concentration recommended for high volume spraying, to ensure sufficient chemical being applied to the trees.

The fact is that optimum strength of the chemical necessary for low volume spraying has not been worked out on a scientific basis, and it may well be that a weaker concentration will provide equally good results. In the meantime it will be of interest to determine effects of the high concentrations of spray chemicals upon the general fauna of the orchard, and upon some of the beneficial species in particular.

To conclude, as the result of research experiences and observations made during the past thirty-five years, it becomes more and more obvious to the writer that the vexed problem of pest control of the orchard will not be solved by the application of chemicals alone. The problem is indeed very complex, and while it is true that spray chemicals will continue to be used, it is necessary that their application be reduced to the minimum and be used

intelligently. For example, much more time should be devoted in the search for selective chemicals, and more knowledge is required to ensure the correct timing of their application.

At the same time, and for preference, the same team of research workers should speed up the ecological study of the beneficial species of insects, mites and spiders, and indeed the whole fauna of the orchard, and in particular those species which are likely to provide a succession throughout the season, in the hope that in due time the harmonizing of chemical and biological control may become a reality.

# Progress in Harmonizing Biological and Chemical Control of Orchard Pests in Eastern Canada<sup>1</sup>

By A. D. PICKETT<sup>2</sup>, W. M. L. PUTMAN<sup>3</sup>, and E. J. LEROUX<sup>4</sup>

## ABSTRACT

Spray programs that harmonize biological and chemical control of apple pests have been successfully developed in Nova Scotia through the use of pesticides that are relatively innocuous to biological control agencies, and the general reduction of insecticidal sprays to the minimum required for immediate control of specific pests. They are now used in most Nova Scotian orchards and have greatly reduced the cost of pest control.

Similar programs have given very good results experimentally in southwestern Quebec.

In Ontario, less progress has been made in developing such spray programs for peach orchards. The only insecticides giving sufficient control of the oriental fruit moth, *Grapholitha molesta* (Busck), to satisfy the exacting market standards of this fruit are DDT and parathion, which have very drastic effects on biological control agencies.

## INTRODUCTION

As a number of papers have been published dealing with the subject matter herein described it seems unnecessary to review the preliminary work in detail (Lord, 1947, 1949, 1956; Lord and MacPhee, 1953; MacPhee, 1953; Paradis, 1956; Pickett *et al.*, 1946, 1953; Stultz, 1955). It will suffice to state that the concept on which the original work was based involves the thesis, as stated by Nicholson (1939), that the repeated use of an insecticide does not necessarily lower, over an extended period, the population level of the pest species against which it is directed. Extensive field experience has shown that while the initial applications of a pesticide may give outstanding results this does not insure that it will necessarily continue to do so. Furthermore, species which were not pests may attain pest status following the application of certain pesticides. A number of factors may account in some measure for these apparent anomalies. Some of these may be outlined briefly as follows:

1. A pesticide may interfere with intra- or interspecific competition.
2. A pesticide may increase the reproductive or the survival potential of a species by influencing its physiology or that of its host (Hueck *et al.*, 1952; Hueck, 1953).
3. A pest species may become resistant to a pesticide.
4. Natural enemies may be reduced in numbers.

Some species, such as phytophagous mites, compete intraspecifically when population levels rise to the point where the physiology of the host plant is seriously impaired. Defoliating insects such as the tent caterpillars, *Malacosoma* spp., and some of the geometrids occasionally consume the available food before reaching maturity. In such cases the application of a pesticide may reduce the pest population to a point where the life cycle of a greater proportion of the individuals is completed, thus prolonging the infestation and causing more severe damage than would have resulted otherwise. That there is interspecific competition is well known.

We have no direct evidence that there is a stimulatory influence of spray chemicals on any species in Eastern Canada, at least as far as apple pests are concerned. Lord (1949) found such a close correlation between the numbers of *Metatetranychus ulmi* (Koch) and its predators that we believe any influence of DDT on the reproductive potential of this species is not of major importance in this area.

There can be no doubt that in some species pesticide-resistant strains have been selected. This is particularly true of species having multivoltine races. This adaptive characteristic of many orchard pest species is a matter of considerable concern to orchard entomologists.

We believe that the destruction of the natural enemies of pests is of major importance in pest population fluctuations and our studies have been largely concerned with this aspect.

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Our studies have dealt with two main problems, first, the long-term influences of specific pesticides on established and potential pests and, second, the factors involved in establishing pest population densities.

We have attempted an ecologic rather than a strictly economic approach.

### STUDIES IN NOVA SCOTIA

Studies on several important apple orchard pests were started in 1943 (Pickett *et al.*, 1946). In general two methods of approach were followed. First, orchards which had not received spray treatments for some years were treated with various spray chemicals. Records were taken on the faunal populations before, shortly after, and at various intervals for some years following the treatments. Second, orchards which had been regularly sprayed for some years were treated with individual pesticides over a period of time in order to study the population changes. When possible, separate, isolated orchards were used to test each individual pesticide. The fact was recognized that regardless of the purpose for which a chemical was used its long-term effects on the fauna must be studied before it could be safely incorporated into the spray program.

When these studies were started in 1943 four pests were of major importance in Nova Scotia orchards, namely, the codling moth, *Carpocapsa pomonella* (L.), the eye-spotted bud moth, *Spilonota ocellana* (D. & S.), the oystershell scale, *Lepidosaphes ulmi* (L.), and the European red mite, *Metatetranychus ulmi*. These had not always been serious pests; the eye-spotted bud moth had a much longer history of extensive damage than the other three. It is known to have been a pest of some importance in the early part of the second decade of this century and continued as a serious menace until 1955. The European red mite became of importance between 1925 and 1930 and the other two between 1930 and 1940. Although it is not possible to assert categorically that the increase in numbers of the latter three was due to a general increase in the use of sulphur sprays, the evidence indicating this is very strong. In any case, with the more or less general adoption of modified spray programs the populations of all four pests have substantially subsided and the codling moth is the only one which may still be regarded as of major importance.

What we refer to as modified spray programs include low-residue fungicides which are more or less innocuous to beneficial arthropods and, if insecticides are necessary, the use of those that are sufficiently specific to substantially reduce the abundance of pest species without unduly reducing the numbers of beneficial ones.

Other pest species that have decreased in importance since modified programs have been generally accepted are the apple aphid, *Aphis pomi* DeG.; the rosy apple aphid, *Anuraphis roseus* Baker; the woolly apple aphid, *Eriosoma lanigerum* (Hausm.); the apple mealybug, *Phenococcus aceris* (Sign.); the green budworm, *Hedia variegana* (Hbn.); the leaf rollers *Amorbia humerosana* Clem., *Pandemis limitata* (Rob.), *Archips persicana* (Fitch), *A. rosaceana* (Harr.), and *Argyrotaenia mariana* (Fern.); and the leaf miners, *Lithocolletis malimalifoliella* Braun and *Nepticula pomivorella* Pack. How many of these species, or to what extent any of them, were influenced by the sprays is not known at present but it seems a reasonable inference that the general increase in predacious and parasitic arthropods which almost invariably followed a reduction in the use of widely toxic spray chemicals has contributed in no small degree to the reduced populations of at least some of the species.

A few pest species which have shown population increases since modified spray programs have been followed include the fall cankerworm, *Alsophila pometaria* (Harr.), and the tent caterpillars *Malacosoma americanum* (Fabr.) and *M. disstria* Hbn. Outbreaks of these pests occur at irregular intervals and are easily controlled by the use of selective insecticides or by special applications. The apple sucker, *Psylla mali* (Schmldb.), is another pest which has increased in many orchards during the period. Our present conjecture is that some of the new organic fungicides suppress the entomophagous fungus *Entomophthora sphaerosperma* Fres. which, it is believed, ordinarily keeps this insect under control.

A few species, such as the fruitworms belonging to the genera *Xylena* and *Lithophane*, exhibited moderate population increases for two or three years following the cessation of the copious use of insecticides as routine procedures but they gradually subsided to normal. In Nova Scotia all species of phytophagous mites, with the exception of some of the apparently non-injurious free-living eriophyids, subsided rather rapidly following the adoption of modified sprays. *Tetranychus telarius* (L.) subsided first, followed by *Metatetranychus*



*ulmi* and finally by *Bryobia praetiosa* Koch. The latter may persist in substantial numbers for as long as three years but all three species may be controlled, if necessary, by the judicious use of selective miticides. Once the populations have been reduced substantially, especially if this is accomplished by predators, they rarely increase to pest status and then only for short periods.

The results of these studies is the more or less general adoption of modified spray programs by Nova Scotia growers who in 1955 produced approximately four million bushels of apples. Probably an average of not more than two applications of insecticides are used per year and the damage from pests in 1955 was lower than at any time in the last 30 years.

### STUDIES IN QUEBEC

The suggestion that the faunal relationships in Nova Scotia apple orchards are unique is not borne out by studies in the Province of Quebec. Here the liberal use of widely toxic pesticides in apple orchards has resulted in a complex of pest problems. There were rapid increases in populations of the European red mite and the red-banded leaf roller, *Argyrotaenia velutinana* (Wlk.), following the use of DDT for the control of the codling moth (Paradis, 1956). Some pest problems which did not previously exist in the province were created (LeRoux and Parent, 1956), and others, previously relegated to a minor role, were intensified. Studies directed toward a modification of the orchard protection program were initiated at the St. Jean Laboratory in 1951.

To determine how the spray programs were influencing faunal populations and how they might be modified to best suit Quebec conditions, a survey of apple orchards was made in the major apple-producing regions of the province. The results showed that such highly toxic pesticides as DDT and parathion markedly reduced the predator populations as compared to treatments with fungicides only or with fungicides plus an arsenical. There were still greater differences between the insecticide-sprayed orchards and those that received no sprays although, in the latter, much of the foliage was destroyed by apple scab. In orchards where DDT had been used for three or more consecutive years, pests of economic importance were on the ascendency.

With this information and the results of the Nova Scotia studies as a background, experimental orchards were established in 1951 at Rougemont. Two 1,200-tree plots were treated respectively as follows: (1) Modified spray program: Regular applications of a preventive fungicide (glyodin), with an insecticide (lead arsenate) and a miticide (Ovotran) used only as correctives; (2) Commercial spray program: Preventive sprays used regularly for the control of apple scab, insects and mites with no attempt to select those innocuous to beneficial species. This is the program given in the Quebec spray calendar and which includes DDT and parathion.

Five years' records are now available from these plots; for the past three years weekly records on pest and beneficial species have been taken during the growing season. A total of 64 pest species were recorded of which only five are regarded as of major importance. A review of the literature reveals that all 64 species have in the past caused heavy damage to apple orchards in North America or elsewhere.

For the five years the modified program plot received an annual average of 9.2 fungicidal and 2.2 insecticidal (including miticidal) sprays at an annual cost of \$0.65 per tree for spray chemicals. This program allowed an increase from a low to a high density of beneficial species and a maintenance of medium to low density of destructive species. The average yield per tree of insect-free apples was 6.4 bushels. The overall control of apple pests by this program for the entire five years was good, and equal to that in the plot where the commercial program was used.

In the commercial spray program plot for the same period an average of 12.4 fungicidal and six insecticidal sprays were applied with an average annual cost of \$1.14 per tree. This program resulted in the practical elimination of natural control agents with the maintenance of a correspondingly high density of some pest species. The average yield per tree of insect-free apples was 5.2 bushels. The overall control of pests for the period was good.

The modified program has therefore given as good control of pests as the commercial program at approximately half the cost. Much of the effectiveness of the former is due to the increased numbers of both predators and parasites which contributed substantially to the

control of pests. Bird predation also contributed an undetermined amount to the success of this program.

### STUDIES IN ONTARIO

Since 1946, investigations on the possibilities of combined biological and chemical control of peach pests have been carried on at the Entomology Laboratory at Vineland Station, Ontario. They have centered largely around the side-effects of DDT and parathion as applied for the control of the oriental fruit moth, *Grapholitha molesta* Busck. The use of DDT has undoubtedly been responsible for the transformation of the European red mite from an occasional pest in certain orchards to an annual threat requiring the routine use of miticides in practically all peach orchards. The exact mode of action is still uncertain; most predators are certainly destroyed by DDT, but it has not been conclusively shown that this effect alone is responsible for mite increase. In non-sprayed experimental plots the populations of both predators and their prey have usually been so low that their relations are obscure.

The response of the two-spotted spider mite, *Tetranychus telarius*, to DDT has been more erratic. Before this insecticide was used injurious infestations were unknown in local peach orchards but very high populations have since occurred in a number of orchards after several years' spraying with DDT.

A demonstrated reduction, through the use of DDT, in parasitism of a cottony scale, *Pulvinaria vitis* (L.), is probably one of the reasons why this formerly uncommon and sporadic pest became much more destructive shortly after DDT was introduced. Outbreaks of the European fruit lecanium, *Lecanium corni* Bouché, are also becoming more frequent, probably for the same reason.

Parathion is now used widely in peach orchards in the Niagara Peninsula, usually in combination with DDT, because it affords cheap and effective control of most insect and mite pests. However, in a few orchards where parathion has been used longest the European red mite has begun to show signs of resistance. A number of pests, particularly scales and mites, also tend to rebound very quickly after they have apparently been reduced to insignificance by parathion. In a DDT-sprayed orchard where the European fruit lecanium had become destructive, two applications of parathion reduced the scale to a level where it could not be detected by routine sampling, yet within three years it had nearly regained its former status.

When DDT, and later parathion, were introduced it was feared that they might eventually, through the destruction of parasites, aggravate the infestation of the oriental fruit moth. This threat has not materialized and parasitism for the past few years has averaged higher than previously (Boyce and Dustan, 1958).

Trials of various insecticides for the control of the oriental fruit moth have failed to reveal any as effective as DDT and parathion but without their defects. The use of these insecticides must therefore continue against the moth, which may exist at a comparatively low level for periods of several years, even when insecticides are not used, but from time to time erupts into unpredictable and very destructive outbreaks. One of the most objectionable features of fruit moth attack is concealed infestation in the fruit, which cannot be culled and has a very bad effect on consumer demand.

Studies on the influence of fungicides, chiefly sulphur and captan, on the fauna have been inconclusive so far. Sulphur definitely suppressed predacious phytoseiine mites, and populations of the European red mite have averaged higher in sulphur-sprayed plots than in captan-sprayed or non-sprayed ones, but injurious infestations have occurred in sulphur-sprayed plots only where they were adjacent to DDT-sprayed ones with intense infestations. Applications of sulphur for brown rot control on peaches are generally fewer and more widely spaced than those applied to apples for scab control. The clover mite, *Bryobia praetiosa*, and the peach silver mite, *Vasates cornutus* (Banks), have increased where sulphur was omitted but injurious infestations have not yet been seen. The effects of fungicides on the fauna will remain largely of academic interest as long as more drastic materials like DDT and parathion are included in the spray schedules.

### CONCLUSIONS

It is obviously unwise to attempt to draw more than tentative conclusions from the available data. Nevertheless, the studies have gone far enough and the results have been

sufficiently consistent to justify the development of working hypotheses which may serve as guides to future studies. These may be enumerated as follows:

1. The use of widely toxic pesticides reduces the populations of beneficial arthropods as well as of pest species. The routine repetition of applications of such toxicants, for the purpose of achieving complete freedom from pests, may establish a new set of environmental conditions depending on the differential influence of the pesticide on all the species involved. The overall result of these new conditions may not be more favorable economically, and in fact may be worse, than the original condition.

2. In the orchards of Eastern Canada some pests now regarded as of major importance would not exceed an economic tolerance level if pesticides did not interfere with natural control agents. Whether this applies to all indigenous and long-established species is not clear at present.

3. That there is an intricate relationship between phytophagous and predacious and parasitic species is obvious. Neither the factors influencing population levels nor the extent of the ecosystem are so obvious.

4. The economics of fruit-growing requires that reliable techniques for the control of pests be established. Whether these are largely of an artificial or of a biological character will depend eventually upon the relative effectiveness and the respective costs. That chemical pesticides invariably cheapen or simplify the control of pests cannot be taken for granted. The initial results obtained from the use of pesticides frequently give false impressions of their ultimate worth. The results of our studies suggest that it may be practical to develop a system of faunal management in orchards which will keep the majority of species below the economic zero without resorting to the extensive use of pesticides which are toxic to a wide range of species.

5. Newly established species of pests and probably some indigenous species will, at times, require the application of artificial controls. To control such species effectively, without disrupting such biological balances as may exist, the development of highly selective pesticides is necessary. In other words, the logical approach to pest control is, in our opinion, to make biological and chemical control additive factors rather than replacing one with the other.

6. The concept of encouraging environmental resistance in orchards may be likened to that of building internal animal resistance in human and veterinary medicine. The latter is now a well established principle which has profoundly influenced the scientific concepts and methods of disease control, and the principles involved may be equally valuable in respect to the control of the arthropod pests of plants.

A result of the acceptance of these tentative conclusions has been the development and general use, in Nova Scotia, of spray schedules based on pesticides that have a considerable degree of specificity or selectivity. Although these have been reasonably satisfactory, there is still much to be desired, and it is our opinion that much progress may be achieved by increased research on host-predator and host-parasite relationships, the extent of the ecosystem involved, the ecological influences of pesticides on the orchard fauna, and the development of more highly selective spray chemicals.

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# The Flexible Approach in Orchard Entomology<sup>1</sup>

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## ABSTRACT

*The Entomology laboratory at Summerland is not committed to one method of insect control, whether biological, chemical or otherwise; nor to the purely academic or purely practical approach; nor to either long-term research or short-term ad hoc work.*

*In biological control there have been some successes, but not against the most serious pest, the codling moth, where, however, DDT has been the salvation of the grower. In case DDT resistance should develop in this species alternative insecticides are being tested; Diazinon has been found to be an excellent alternative. The development of automatic concentrate sprayers has been a major part of long-term work on practical control and has long passed the pioneer phase so that improvements are now mainly an engineering matter. A new long-term study in practical control lies in an effort to control codling moth by the release of males sterilized with radioactive cobalt. The study of the ecology of several species of orchard mites and their predators in relation to orchard environment is a main effort in long-term biological studies. These reveal great differences from the situation in other parts of Canada. Short-term studies designed to keep the spray calendar of B.C. up-to-date are an important part of the work of the laboratory and are not regarded as of lesser status, or needing lesser skill and judgement, than other projects. In fact a very heavy responsibility lies on those who introduce changes in the calendar as the orchardists of B.C. are quick to seize on new methods so that a faulty recommendation could have serious consequences.*

The function of the Entomology Laboratory at Summerland is to provide solutions for the insect pest problems that beset the orchardist of the interior of British Columbia. We believe that a flexible approach is the best way of carrying out this duty. This does not mean that we change our work or policies at short notice. It means, however, that we do not wish the work of the laboratory to be strongly biased in any one direction: neither to long term research nor short term work; nor to fundamental work nor *ad hoc* applied work; nor to one doctrine of control methods, whether it be biological, chemical or cultural. At a university laboratory, or in a similar institute a strong orientation of effort in one direction by the whole staff may lead to outstanding advances; but our duties are different from those of a university laboratory and we cannot put the bulk of our effort in one direction without neglecting some of our obligations. In connection with these obligations our work includes (a) short-term studies designed to alleviate current insect problems; this work is on a year-to-year basis and the results used in our annual re-writing of the British Columbia spray calendar; (b) long-term, more fundamental studies designed to solve difficult and important insect problems for which current control methods may be unsatisfactory or expensive; (c) investigations to discover the reasons for changes in the insect or mite fauna of our orchards over periods of years; we believe, incidentally, that we have been much too prone to blame such changes on the effects of new insecticides in destroying parasites or predators; (d) both short-term and long-term work on the design and improvement of air-blast concentrate spraying machines, and on the elucidation of the aerodynamic, physical and chemical principles of this method of spraying; (e) investigation on entirely new methods of control where they appear appropriate for our more serious pests.

Little more than a generation ago, applied entomology in this country frequently meant little more than collecting insects, preparing demonstration mounts or conducting perfunctory experiments in the field—experiments which sometimes lacked adequate controls, and may have been unreplicated either in space, or in time. In the face of rising losses from insect damage, that approach became inadequate. And as university training in entomology came to include more and more physics, chemistry and mathematics and particularly statistics, the casual days of applied entomological research came to an end. The demise of the old methods might be deplored by some, but there can be no question there had to be a change. Now, however, we are in danger of moving too far in the other

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direction. Sometimes one detects a disturbing tendency to mistake the means for the end, and as John Stuart Mill once said about a phase in his own studies, to move with a sail but no rudder.

What we might call the theoretical approach unquestionably has done much for orchard entomology. Experimental results, generally speaking, have greater significance nowadays than twenty-five years ago. But, in fairness to the pioneers, where in their time one man might work on a problem unaided, nowadays, on the same sort of problem, we expect to find a project leader and several assistants, and a battery of mechanical aids. In some respects, the methods of conducting field experiments today could have been followed yesterday. In spraying experiments, for example, more attention could have been paid to the use of compounds of known composition rather than trade products with proprietary names. Controls might have been given greater emphasis and, in many instances, sampling methods could have been improved without adding to the work.

On the other hand, much of today's detailed ecological work would have been impossible for our predecessors. It involves little that is new in technique, but requires a large staff. Most of the statistical procedures now in vogue are comparatively new and they require equipment only recently available to the researcher. The calculation of statistical odds, a common job nowadays, takes more time than the lone worker a generation ago could have spared.

Occasionally we notice a somewhat apologetic attitude towards the experiments of the pioneer in applied entomology. Perhaps it arises from unfamiliarity with the problems he had to face, or his limited facilities, or from failure to appreciate that he did a fairly good job of helping to keep the agricultural economy of his day at a profitable level. But it seems equally probable that it may arise from an unbounded faith in statistical procedures (applied by entomologists who rarely understand the underlying probability theories) and because, within the last few years, it has been common thinking to decry short-term investigations as mere testing, and to laud long-term, fundamental research.

We may make use of an analogy from farming to illustrate our thinking on this subject. In this age of mechanisation, and especially in Canada, the tractor and all manner of agricultural machines have replaced human and animal muscles on the farm. But the spade and the hoe—implements invented in prehistoric times—still have a place on the most modern farm. In fact they are indispensable for many jobs; on work for which the tractor and its attachments are unsuited, or where it would be ridiculous to use them. Such, also, is our outlook on field experiment. For most entomological work today flawless statistical procedure and design is essential or highly desirable. But there is still work, of the spade and hoe kind, where the time-honoured and simple methods are more suitable and appropriate; and where it is of little or no practical value to spend an inexcusably long time using some very precise method of design, or of evaluation of infestation, or of analysis of results.

Much of our investigations in connection with the annual revision of the spray calendar falls into this category. The spray calendar of British Columbia makes reference to some 40 species of insects and mites. Apart from a hard core of serious offenders, by no means all are prevalent every year. In any one year, therefore, only limited opportunities may be available for testing promising new insecticides against many species; species that may be very troublesome the following year when information will certainly be wanted and expected by the grower. Time, or orchard conditions, do not then always permit a perfect layout of experimental plots. However, as much—and as varied, rather than as precise—information as possible must be obtained. Fortunately, since we are concerned only with very large and obvious differences of control efficiency in this work, the effectiveness of a particular material can be gauged without recourse to elaborate statistics.

Since the introduction of synthetic organic insecticides, we are faced every year with testing the effectiveness of many new chemicals against several species of insects and mites. The primary purpose of this work is to determine, as quickly as possible, which chemical should be recommended to the fruit grower for pest control. Two things have to be remembered at all times; we are looking for large differences in effectiveness, and even though a chemical may be very effective, certain practical considerations will determine whether it can be recommended. Precise methods of estimating population densities are a primary requisite in ecological problems. But in "short-term" chemical experiments where we are looking for large differences in effectiveness, a great expenditure of time on precise measure-

ments or analyses cannot be justified. For example, many investigators determine the effectiveness of aphicides by counting, before and after spraying, the number of living aphids on a sample of leaves. We have found, however, that we can obtain reproducible results by using much more rapid methods than those involving actual aphid counts. In one of these methods we examine five terminal leaves on an infested twig and record the percentage of these leaves that are free of living aphids. An apparent weakness in this method is that a leaf with one or two living aphids is given the same rating as a leaf with 100 or more living aphids. But this is not a serious shortcoming; when conditions in an orchard are favourable for the multiplication of aphids they reproduce so rapidly that even a very low initial population will reach injurious level within one or two weeks. Consequently, unless an aphicide gives almost perfect control, it usually should not be recommended.

Orchard experiments with spray chemicals must of necessity be kept flexible. The orchards in the Okanagan Valley are mostly small. Replication within the orchard is usually impossible. One-tree replicates may be suitable under certain limited conditions, but more frequently so many buffer trees would be required to reduce spray drift and reinfestation that replication again becomes impractical. On the rare occasion when we have an orchard sufficiently large to permit replication we must still keep the flexible approach and be prepared to alter our experimental design at short notice. For instance, just as we are going to commence spraying on some fruit grower's property the wind might start drifting from an angle that would cause excessive spray movement from one plot to another. Immediately we have to use a new plot design one which will lessen the influence of the wind, even though the new design may not give us as much information as we had originally hoped for. Some may ask, "Why not delay the experiment until the wind stopped blowing?" Very frequently this cannot be done. Delay may result in serious crop loss from insect damage; the insect may reach a stage of development in which it is difficult to control; or the tree may reach a stage of development in which it will be injured by the chemical.

Even where orchards are large enough to permit replication within the orchard, the experiment has to be repeated under different conditions before we can be reasonably certain that the chemical can be recommended for use throughout a valley in which climatic and other factors may vary greatly from one orchard to another. Take a simple example. As the result of one replicated and statistically analyzed experiment we find that nicotine sulphate gives significantly better control of aphids than the other chemicals tested. However, if we repeat this experiment in another orchard, on a day when the temperature is fairly low, we might very well find that nicotine sulphate is obviously less effective than the other chemicals. With new chemicals we have no idea how temperature, rainfall, and many other uncontrollable variables will affect the killing power or the phytotoxicity of the chemical. Consequently, small experiments, individually imperfect perhaps, repeated in several orchards under different conditions, particularly of weather, are preferred to large-scale experiments of great statistical precision limited to one or two orchards.

With such thoughts in the back-ground, entomological research is planned and carried out for the fruit industry of British Columbia. The problem of controlling the codling moth, the worst enemy of our fruit growers, will illustrate. The codling moth has developed a pronounced resistance to DDT in South Australia (Smith, 1955) and Ohio (Cutright, 1954). Since in time the same phenomenon probably will occur in British Columbia, it is logical to prepare for it. Obviously the British Columbia fruit grower cannot revert to the relatively ineffective codling moth insecticides of the pre-DDT days. Work in the United States, Canada and elsewhere has shown that in two- or three-generation areas, little help against the codling moth can be expected from parasites or predators. What may be needed is an insecticide at least as toxic to the codling moth as DDT but of distinctly different chemical configuration. Two years ago, preliminary research suggested that the organic phosphate Diazinon might meet requirements. Arrangements were made to determine its effectiveness against the codling moth.

Diazinon might have been applied to a commercial orchard in comparison with DDT in the usual number of spray treatments. But in most commercial orchards, because of currently effective spraying with DDT, the codling moth exists only in trace proportions, and its numbers are greatly influenced by such features as topography of the orchard and proximity of buildings, roads and open areas, irrigation flumes, prop piles, box piles, and



headlands. Commercial spraying in British Columbia is almost exclusively done by automatic concentrate sprayers. Even in the case of reasonably high infestation it is not feasible, with such equipment, to spray the many replicates that would be involved in a satisfactory statistical design.

This problem typical of a field laboratory, was attended to by working with a high infestation and adapting equipment to orchard. A small, abandoned orchard was used in which, year after year, the fruit had been almost entirely destroyed by the codling moth. It was sprayed by a dual-tank, high-volume, high-pressure, mobile sprayer. One tank was charged with a DDT suspension, the other with a Diazinon suspension. Successive pairs of trees of the same variety were sprayed with each of the compounds. Only three sprays were applied in order to ensure a moderately heavy codling moth infestation. At harvest time, the infestation was recorded on each of the seven, two-tree plots, sprayed with each compound. Adjoining plots had reasonably similar infestations; the average infestation approximated 12 per cent for each material and 80 per cent for the non-sprayed controls. Obviously Diazinon was approximately as effective as DDT.

But this does not mean that Diazinon will invariably be as effective as DDT. The growing season was abnormally dry and cool. No matter what the statistical odds, they might have been quite different had the season been wet or hot. Diazinon, or any other new orchard insecticide for that matter, can be properly evaluated only after it has been under trial for several years. A modicum of work with Diazinon against codling moth will then be carried out in the next few years though the material is unlikely to be needed unless DDT-resistant codling moth appears. Stylized thinking has no place in this kind of work. What is needed is the flexible approach and a doubting mind that demands *obvious* similarity or difference, and *repeated* demonstration. Even if orchards permitted, which, in the majority of cases they do not, repeated experimentation of a type to satisfy the statistical idealist could only be undertaken at unreasonable cost. On the other hand, the welfare of thousands of fruit growers must not be prejudiced by limited trial, no matter how nearly perfect the individual experimental design.

In planning research on the control of orchard pests, or in making recommendations, a reasonable balance should be maintained between the various control procedures. To forget that cultural practices, which are currently changing very rapidly, may influence the abundance of insects and mites may result in an unwelcome influx of species which ordinarily might be unobjectionable. To depend exclusively on chemicals, particularly in this day of numerous physiologically potent synthetic compounds, may lead from one pest outbreak to another, and consequent increases in production costs. On the other hand, the opposite view might bring even greater difficulties. Strong reliance on biological control may encourage a mental withdrawal from the hard realities of making a living from the soil. In British Columbia, the orchard entomologist must never forget that, in most cases, orcharding offers no more than a modest income. The fruit grower cannot afford to gamble that Nature will solve his insect problems if she is left alone; to imply that he can is to invite the loss of his confidence. And that eventuality the official agriculturist should avoid like the plague. Without the confidence of the people he is employed to serve, he can never be fully effective.

Little more than a decade ago the cost of controlling insects and mites in the orchards of British Columbia was so great that it threatened the very existence of the fruit industry. Added to high irrigation, service, and labour costs, the bill for spraying would have been much too great even if it had guaranteed good fruit. But, too often, the loss in terms of damaged fruit was as high as the cost of spraying. The total cost of insect attack to apple orchards ranged from 15 to 30 cents per box, about the highest in the business. Today, thanks to new chemicals and new spraying technique, the figures are 5 to 10 cents per box, about the lowest in the business. This revolution in pest control, for that is what it proved to be, has meant the difference between ruin and reasonably comfortable survival for thousands of fruit growers.

The welcome change in the fortunes of the British Columbia fruit industry was brought about quite simply by the application of ordinary logic and steady work. The chemical industry had produced powerful new synthetic insecticides and acaricides. Repeated short-term experiments showed that some of them were exceedingly effective and reasonably safe to use. Commercial operations showed that these compounds could be



depended upon to reduce the most serious pests to trace proportions. But the cost of labour and materials for so doing were still much too high. The small and hilly orchards characteristic of British Columbia's southern interior precluded the use of the large and expensive but labour-saving air-blast sprayers that had just been developed in the United States. Obviously spraying operations had to be mechanized, but how?

Further experiment showed that all the new spray chemicals and most of the older ones could be applied at increased concentration and decreased gallonage without measurably affecting their insecticidal or acaricidal efficiency. If it were possible to apply such concentrated mixtures by some sort of mechanical device which operated continuously along the rows as did the large high-volume air-blast machines, obviously it might be relatively small and presumably relatively cheap to buy and operate. And being small, it could be hauled by the light, wheeled tractor owned by almost every fruit grower. Fortunately, wartime engineering had produced two machines which simplified the problem. One was a steam-operated fog-maker<sup>2</sup> which, at the turn of a switch, could project spray droplets as fine as fog or as coarse as rain. The other was a small but efficient air-turbine. By combining the two, adding a small high-pressure hydraulic pump and a light but fairly powerful gasoline engine, and mounting the whole on rubber-tired wheels, an experimental concentrate sprayer was born. That machine, known as the Okanagan experimental sprayer, demonstrated the approximate optimum drop size for concentrate spraying, and the approximate optimum gallonage per acre. Then, in orchard after orchard, first on one type of insect or mite and then on another, and finally on apple scab, it demonstrated that concentrate spraying was a sound practice for the fruit grower. A year later three manufacturers had undertaken to produce light and relatively low-cost concentrate sprayers modelled after the prototype experimental machine. Within five years the spraying operations of virtually the entire British Columbia fruit industry had been mechanized by concentrate sprayers and the industry was perhaps more prosperous than at any time in its history. A more detailed account of the development of these methods will be given in a separate paper to this Congress (Marshall, 1958).

Here was another practical demonstration of the flexible approach in applied entomology. If extensive statistical work, biological control measures, or detailed ecological studies had been indicated, appropriate work would have been attempted. But in that event an increased staff would have been necessary and almost certainly the practical outcome would have been considerably delayed. The methods that were used, though unpretentious, were quite adequate. They did the job to the entire satisfaction of an industry in serious trouble, and they did it quickly.

Lest it appear that the operations of the Summerland Laboratory take no account of long-term investigations, it should be mentioned that about thirty per cent of the entomological research is devoted to fundamental work on orchard mites, most of which is ecological. This work is being reported at this Congress by Anderson and Morgan (1958). Approximately twenty per cent of the research is on life-history work.

However successful our current methods we must not become complacent. Insects can fight back powerfully, and not least among their weapons is the genetic variation, or short-term evolution, that leads to chemically resistant forms. DDT resistance in the codling moth in British Columbia is at present only a threat for which we are at least partially prepared. However in orchard mites, particularly the European red mite, *Metatetranychus ulmi* Koch, (Downing, 1954), resistance to parathion, malathion, and other organic phosphates has developed very rapidly in several areas of British Columbia. Several specific miticides are very effective in such cases; but such miticides, as a group, are prone to cause foliage and fruit injury to certain varieties. Nowadays, therefore, the search for a miticide that can be recommended to growers is no more a search for miticidal efficiency than for an absence of phytotoxic effects (Downing, 1957).

Not only must we avoid complacency but also conservatism. The best methods of today will inevitably become obsolete. A long-range project on which work has commenced at Summerland is on the possible control of codling moth by the mass release of males that have been sterilised with cobalt-60, a method which has been used recently to exterminate the screw worm in Curacao (Baumhover *et al.*, 1955). The chances that we can exterminate the codling moth by this means are probably slight; but we would be short-

<sup>2</sup>Besler Engineering Corporation, Emeryville, California.

sighted if we did not explore the possibilities of any method of control that represents a new approach to the problem.

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# New Possibilities in Apple Pest Control

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## ABSTRACT

The increasing complexity of apple-pest problems and spray programs despite the use of the powerful modern pesticides has clearly indicated the need for a better understanding of the causative factors. Outbreaks are often attributed to the destruction of natural enemies of insect pests by these materials. A study has therefore been made in the Shenandoah Valley to determine the importance of the beneficial species and the extent to which they are affected by various pesticides. The least harmful spray materials have been used in selective spray schedules to achieve a closer integration between chemical and biological control.

The fungicides glyodin and captan and the botanical insecticide ryania were found to be relatively harmless to mite predators. In orchard tests ryania was nearly equal to DDT against the non-resistant codling moth, but gave superior control of DDT-resistant strains. Ryania has not injured plant tissues, is comparatively safe for man and warm-blooded animals, and is one of the few pesticides exempt from residue tolerance on growing crops.

We are all familiar with the miraculous control of the codling moth (*Carpocapsa pomonella* (L.)) that followed the adoption of DDT about 10 years ago, and with the destructive outbreaks of orchard mites, red-banded leaf rollers (*Argyrotaenia velutinana* (Wek.)), and other formerly minor pests that followed in its wake. These outbreaks necessitated a desperate search for new pesticides. Although even more complex and powerful materials have been found, the search has been only partially successful, as indicated by the continued importance of these problems.

The increasing complexity of apple spray programs despite the use of infinitely more effective modern pesticides is the most baffling enigma yet faced by orchard entomologists. In New York State, for example, as many as 20 spray treatments at an annual cost of about \$100 an acre are now required for the control of apple insects and diseases (Chapman 1955). These programs often include a dozen or more different materials, sometimes combining four or five in a single application, and their cost continues to mount.

Now we are threatened with two very serious new problems—the development of DDT-resistant codling moths and the danger of exceeding the legal tolerances for pesticide residues on harvested fruit.

Although DDT still gives satisfactory control of the codling moth in most orchards, many eastern growers have had to resort to heavier dosages and extra sprays, often supplemented with parathion. If continued, this practice will obviously lead to a residue problem and may hasten the development of resistance. Apparent resistance has already been reported from several States, and in view of earlier experiences with lead arsenate and the widespread development of DDT-resistance in many other insects, there is little hope that the codling moth will be an exception.

In our attempts to “sterilize” the orchard environment, we seem to have underestimated the tremendous recuperative and adaptive powers of our arthropod enemies, as well as the importance of our arthropod allies. A better understanding of the fundamental ecological and physiological aspects of these problems is obviously required for their solution.

Recent studies of this type, as reviewed by Ripper (1956), indicate that pest outbreaks following chemical treatments are usually due to one or more of the following causes: (1) reduction of natural enemies by toxic pesticides, (2) development of pesticide-resistant strains, (3) stimulation of pest development through favorable influence of the pesticide, and (4) removal of competing pest species. A combination of several causes may produce the greatest disturbance, as has apparently occurred with the orchard-mite problem.

Another factor, which is frequently overlooked and may be highly important, is the omission or reduced usage of pesticides that formerly helped to suppress the species in question. In eastern apple orchards it now appears that lead arsenate was chiefly responsible

<sup>1</sup>Resigned September 6, 1956.

for holding red-banded leaf rollers and plum curculios (*Conotrachelus nenuphar* (Hbst.)) in check, while the general use of summer-oil stickers undoubtedly contributed to the control of European red mites (*Metatetranychus ulmi* (Koch)). Better condition of the foliage with modern spray and fertilization programs may also have favored mite increase.

An ecological study of the factors causing these disturbances was begun in West Virginia in 1952, with major emphasis on the evaluation of natural enemies and the possibility of integrating chemical and biological controls through selective spray programs featuring materials that are relatively harmless to the beneficial species. Since earlier studies had shown that even before the advent of DDT biological control of the codling moth was unsatisfactory in areas where it has more than one annual generation, our greatest need was for an insecticide having most of the advantages of DDT without its harmful effect on the beneficial species. For use in complete spray programs we also needed an effective fungicide with selective qualities that might permit increased biological control of other apple pests and thus reduce the need for special pesticides.

In a recently abandoned orchard the botanical insecticide ryania and the organic fungicides glyodin and captan showed little or no effect on mite predators, and damaging mite infestations failed to develop on trees receiving seasonal schedules of these materials (Clancy & McAlister 1956). Glyodin also showed an early-season effect on the European red mite not possessed by captan. The use of DDT and sulfur fungicides on other trees virtually eliminated predaceous mites of the genus *Typhlodromus* and resulted in heavy red mite populations.

Ryania has consistently given outstanding control of the codling moth in both abandoned and commercial orchards comparable to that usually obtained with DDT, when used at the rate of 5 or 6 pounds in 100 gallons of water with glyodin at the same intervals recommended for DDT. It was somewhat less effective when used alone, with captan, or at lower dosages, but wormy apples were scarce in all ryania plots. Most of the injury consisted of very small, superficial stings. An activated formulation was not enough better than ryania alone to justify its higher cost and was more destructive to the beneficial species.

The main active principle of ryania is the powerful alkaloid ryanodine, which paralyzes insect muscle tissue by its action as both a contact and a stomach poison (Heal 1949). Comparatively high dosages are required, since the ryanodine content of the ground stem is only about 0.025 percent—an indication of its extreme toxicity to the codling moth as compared with that of DDT. Ryania is comparatively harmless to warm-blooded animals, does not injure plant tissues, has high stability, and is one of the few pesticides exempt from a residue tolerance on growing crops.

The results of annual harvest examinations in ryania, DDT, and water-sprayed check plots in the abandoned orchard are shown in Table I. The failure of DDT to control the codling moth satisfactorily was quite unexpected, and indicates a resistant population. Although a defective formulation contributed to the poor control in 1952, control with a known high-quality brand continued to be unsatisfactory thereafter. Each year most of the damage was caused by larvae of the second generation during August.

Table I also shows the wide variation in European red and predaceous mite populations in these plots. Many of the predators were killed by the activated ryania used in 1952, but populations increased after ryania alone was adopted in 1953. Note the declining red mite infestations in the ryania and check plots, where the predators were unharmed, as compared with the generally opposite situation in the DDT plot, where the predators were destroyed.

Other pests that have been more abundant in the DDT plot include *Tetranychus* mites, the clover mite (*Bryobia praetiosa* Koch), Forbes scale (*Aspidiotus forbesi* Johns.), the unspotted tentiform leaf miner (*Callisto geminatella* (Pack.)), the apple aphid (*Aphis pomi* DeG.), the rosy apple aphid (*Anuraphis roseus* Baker), and the woolly apple aphid (*Eriosoma lanigerum* (Hausm.)).

We are also conducting a long-range study of ryania control programs in four commercial orchards where all sprays are applied by the growers. Seven 1-acre plots of six apple varieties are now receiving the ryania schedule with glyodin as a fungicide, in comparison with standard DDT schedules applied on the same dates to the adjoining trees. The results thus far are shown in Table II. It will be noted that ryania was nearly equal to DDT



TABLE I. Codling Moth Injury and Seasonal Mite Populations in Three Plots in the Abandoned Chew Orchard, Kearneysville, W. Va., 1952-55.

Plot and Year	Codling moth injury			Mites per 100 leaves*	
	Number per 100 apples		Percent of fruit affected		
	Entries	Stings		European red	Predaceous
Ryania					
1952	0	10.0	8.7	248	5
1953	0	19.0	15.6	136	91
1954	0	2.9	2.8	70	76
1955	0	7.0	6.8	15	15
DDT					
1952	107.4	42.7	72.5	212	6
1953	27.4	40.9	42.1	1445	4
1954	7.8	8.7	15.5	2072	3
1955	37.6	75.5	61.1	1136	1
Check					
1952	85.4	0.1	59.7	199	19
1953	84.9	14.7	62.1	146	207
1954	17.6	1.2	16.6	19	164
1955	50.1	20.8	49.9	2	178

\*Average seasonal populations (including eggs) from 10 biweekly counts per plot, May to September.

TABLE II. Results of Codling Moth Spray Experiments in Four Commercial Apple Orchards, West Virginia, 1953-55.

Orchard and Variety	Year	Number of injuries per 100 apples				Percent of fruit affected	
		Entries		Stings			
		Ryania	DDT	Ryania	DDT	Ryania	DDT
Sandy Ridge: Delicious	1953	1.1	0.4	7.6	2.8	7.2	2.9
	1954	7.1	3.8	4.4	1.3	10.5*	4.6
	1955	2.2	0.2	3.7	0.2	4.9	0.4
Grimes	1954	0.4	0	1.9	0.2	2.2*	0.2
	1955	0	0.2	0	0.1	0	0.3
York	1954	0.3	0	0.6	0.1	0.8*	0.1
	1955	0.1	0.2	0.5	0.1	0.6	0.2
Twin Ridge: Black Twig	1953	0.1	0.2	10.5	2.3	9.2	2.2
	1954	0	0.1	0.1	0.1	0.1	0.1
	1955	0	0.1	1.8	0.2	1.8	0.2
Norwood: Gold. Dcl.	1954	0.4	0.2	1.0	0.1	1.3	0.2
	1955	0	0	1.2	0.2	1.2	0.2
Rome	1954	0	0	0.1	0.1	0.1	0.1
	1955	0	0	0.2	0.1	0.2	0.1
Springvale: York	1955	1.6	17.2	39.1	47.5	30.6	42.1**

\*Last cover spray omitted.

\*\*Parathion included in 4 cover sprays.

against the codling moth in the first three orchards, except for a few more small stings in the ryania plots, but was superior to DDT-parathion in the Springvale orchard where spray coverage was less complete. The crop there was so wormy that numerous larvae and pupae were collected in July and given to W. S. Hough of the Winchester, Va., Experiment Station to test for resistance. The results of his tests with their progeny, as given in Table III (series I and II), show that this strain was much harder to kill than the standard Virginia strain.

The results of similar tests with codling moth larvae from the abandoned Chew orchard are shown in series III of Table III. Only six trees have received the standard DDT

TABLE III. Effect of DDT on Codling Moth Strains from Several Orchards. Data Furnished by W. S. Hough from Insectary Tests at Winchester, Va., 1955.

Orchards	Number of larvae used	Pounds of 50% DDT WP per 100 gallons	Percent survival	Total injuries per 100 apples
Series I:				
Virginia	1116	0.5	0.4	1.1
Springvale, W. Va.	826	0.5	33.4	42.5
Series II:				
Virginia	888	0.75	0.7	1.5
Springvale, W. Va.	916	0.75	32.2	38.9
Series III:				
Virginia				
Sprayed	567	0.5	1.8	6.2
Unsprayed	540	0.5	1.8	4.2
Chew, W. Va.	465	0.5	4.3	14.2

schedule each year, and many of the overwintering larvae were of necessity collected from unsprayed trees for these tests. Had nonresistant codling moths been eliminated by treating the entire orchard, as in commercial practice, these differences would probably have been closer to those obtained in the Springvale orchard. The superior performance of ryania in both these orchards suggests that it may provide an excellent defense against DDT-resistant strains if needed.

Legal tolerances for chlorinated hydrocarbon residues on harvested fruit will also limit the use of DDT for the control of second-brood codling moths in many orchards. The greatest problem will arise on early varieties, where spraying is required to within about 6 weeks of harvest, and on mixed plantings of peaches and apples. Although parathion is an excellent substitute for DDT in many respects, ryania may have certain advantages in these situations because of its longer residual activity, lack of human toxicity and plant injury, and exemption from residue tolerance. The substitution of ryania for DDT in the last one or two cover sprays may help to solve these problems. The addition of parathion to about half the usual 5- or 6-pound ryania dosage is another untried possibility.

Since ryania is no more effective than DDT against the red-banded leaf roller and the plum curculio, supplemental materials are required for the control of these pests. The inclusion of lead arsenate in the petal-fall and first-cover sprays of the selective ryania program has given satisfactory curculio control with only temporary suppression of the predaceous mites, but has allowed increased injury from second- and third-generation leaf rollers where TDE was omitted because of its extreme toxicity to the predators. There is yet little evidence of increased biological control of the leaf roller in the ryania plots, and this is presently our most urgent problem.

Although predaceous mites have already shown some increase in several of the commercial-orchard ryania plots, apparently controlling the European red mite by midsummer in one orchard during the last three seasons, the final outcome is not yet apparent.

These investigations have stimulated wide interest in the use of ryania, and the preliminary results of most other fruit workers in the United States and Canada are in general

agreement with ours. Ryania is also reported as highly effective against apple leafhoppers and a leaf miner on pears, and has given partial control of the eye-spotted budmoth (*Spilonota ocellana* (D. & S.)), spider mites, the clover mite, the apple and the woolly apple aphids, tent caterpillars (*Malacosoma* spp.), and *Neolygus communis novascotiensis* Knight, known in Nova Scotia as the green apple bug (Madsen 1956, Patterson & MacLellan 1954). Control of the latter group may also be improved by greater survival of their natural enemies under the ryania program. It has shown little or no effect on the European red mite, the apple maggot (*Rhagoletis pomonella* (Walsh)), or the unspotted tentiform leaf miner on apples.

Ryania is the recommended codling moth insecticide in Nova Scotia, where most growers are following a modified spray program designed to harmonize biological with chemical control (Patterson & MacLellan 1954, Pickett 1955). The promising results being obtained clearly indicate the feasibility of this approach and the need for expanded research of a similar nature in other areas. Bartlett (1956), Clausen (1954), English (1955), and others have discussed the limitations and undesirable effects of the modern wide-range contact-residual insecticides and the advantages of more specific or selective materials that allow greater survival of natural enemies. In view of our present difficulties we cannot afford to overlook any possibilities that may help to solve these problems.

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# Application of Ecological Information to Control of Citrus Pests in California<sup>1</sup>

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## ABSTRACT

*Instances are discussed where ecological information has been or may be used to improve the efficiency of citrus pest control. The classical application of introduction and establishment of new natural enemies needs no elaboration. Aside from this primary phase of biological control, knowledge of direct and/or indirect effects (1) of weather variations on host and parasite population interaction, (2) of application of insecticides on natural enemies, (3) of interaction with other organisms, (4) of cultural practices, (5) of maladjustments between parasite and host biology, etc. has pointed the way to actual or possible improved biological control through (1) periodic colonization of parasites, (2) development of improved strains of natural enemies, (3) use of alternate host plants and/or host insects, (4) changes in insecticide timing, dosage or material to conserve natural enemies, (5) control of ants, (6) modification of cultural practices, (7) and possibly even inoculation of the trees with the pest itself in order to effect control.*

*In chemical control, ecological knowledge of effect of weather and seasonal variations on pest populations as well as on the efficiency of control has given rise to better recommendations and results. The same applies to differences between areas. Recognition of the adverse effects of insecticides on natural enemies along with new recommendations has avoided upsets. Detailed knowledge of pest biology, most susceptible stages and times for insecticidal treatment, etc. has produced, or appears capable of producing, better pest control.*

## INTRODUCTION

In recent years various investigators have been acquiring basic ecological information concerning the entire faunal complex on citrus trees. Such information was largely unavailable during the earlier years of citrus culture (1) because of the immediate necessity of controlling damaging pests by any means at hand, (2) because of the lack of trained personnel, and (3) because basic concepts of population regulation (that is, of pest control) were not so well developed or understood as they are now. As a result, pest control, both biological and chemical, developed more or less empirically, although with notable successes in response to immediate needs.

Biological control procedures usually involved the introduction and colonization of natural enemies in the hope of achieving complete control. Successes have been of immense economic importance to the industry, but failures, and the reasons underlying them, were somewhat ignored. Major pest control operations remained necessary in all areas.

Chemical control usually involved the choice of an insecticide which gave greatest immediate kill of a particular pest and did not harm the trees. Effects on the faunal complex were usually not considered. As time passed, set chemical control programs developed, and in many cases treatments were made on an insurance basis rather than in response to pest population levels, and usually without regard to beneficial members of the faunal complex or long-term trends. Some minor pests developed into major pests, some pests became less abundant, and some pests became harder to control with chemicals. The reasons underlying these changes went, for the most part, uninvestigated. In the period from 1945 to 1956 the cottony-cushion scale, *Icerya purchasi* Mask., forgotten as a pest since 1890, when it was controlled by the introduction of the vedalia beetle, *Rodolia cardinalis* (Muls.), and *Cryptochaetum iceryae* (Will.) from Australia, again achieved the status of a major pest in a California citrus area following the use of DDT. This possibly marked the end of an era.

Even during the past ten years, the status of citrus pest control has been about as follows. Biological control of a few previously serious pests was complete and reliable. Chemical treatment was considered necessary for major pests once or twice annually, and for certain minor pests annually, or as they became serious. In general, both major and

<sup>1</sup>Paper No. 1007, University of California Citrus Experiment Station, Riverside, California, U.S.A.

minor pests were thought to have inadequate natural enemies; hence the only remedy presumably lay in regular insecticidal treatment.

Detailed ecological studies begun in 1945 have indicated that commercial pest control often can be modified, reduced, or in some districts even eliminated, with resulting improvement in control. Findings, as might be expected, have been quite different in various citrus-growing areas. All of these possibilities of improvement in control, aside from the development of certain new insecticides, have stemmed from basic ecological studies of the pests and their interacting complex of host plant, natural enemies, climate, and man-caused cultural and chemical modifications of the environment. Practical application of these findings is, with certain notable exceptions, still in the future.

### ACQUISITION OF BASIC INFORMATION

Knowledge concerning the field population ecology of the citrus faunal complex throughout the range of citrus in southern California was obtained in several ways. Acquisition of census data, followed by correlation of population trends of pests in relation to natural enemies, climate, and host plants, supplied vital information. Experimental tests, which involved comparison of population trends in plots receiving commercial pest control with trends in plots receiving no treatment and/or having certain other components of the environment modified, supplied the most definite demonstration of the influence of various factors on the pest population. Careful field observations often supplied valuable leads. Laboratory studies complemented certain phases of the field work.

Details concerning this work may be found in papers by Smith & DeBach (1942), DeBach (1946, 1955), DeBach, Dietrick & Fleschner (1949), DeBach, Fleschner & Dietrick (1951), and by Fleschner (1952).

### STUDIES IN UNTREATED GROVES

The first step toward an understanding of the complex faunal relationships on citrus was to learn the potentialities of the entire natural-enemy complex in control of all pests in the complete absence of chemical treatments and to analyze the effect of insecticidal treatments on the balance of arthropod populations.

After it was determined what natural enemies could do in the absence of treatment, it became possible to evaluate their intrinsic weaknesses and to determine other environmental factors adversely affecting natural enemy populations. This information led to the exploration of various possibilities of increasing the effectiveness of established natural enemies as well as of reducing the adverse effects of insecticidal applications.

### FACTORS ADVERSE TO NATURAL ENEMIES

From studies in untreated groves it was learned that factors adverse to natural enemies included ants, air-borne dust, climatic extremes, and lack of synchronization in development of host and parasites. Ants, particularly the Argentine ant, *Iridomyrmex humilis* Mayr, were frequently found to be the cause of upsets in biological control. Although it had long been recognized that ants protected "honeydew" producers and thus caused increases in populations of soft scales, mealybugs, and aphids, they were also found capable of causing striking increases in populations of diaspine scales and mites as a result of killing or disturbing natural enemies of these pests in which they had no interest whatsoever (Flanders, 1945; DeBach, Dietrick & Fleschner, 1951). Efficient ant control therefore became a prerequisite for the attainment of over-all effective biological control, and a real help in over-all pest control even under conditions of regular major commercial pest control operations.

Deposits of air-borne dust on citrus trees were found to cause considerable retardation of natural-enemy population growth. Serious upsets are not nearly so general or frequent as those caused by ants, but nevertheless are commonly encountered, especially with populations of the California red scale, *Aonidiella aurantii* (Mask.), and citrus red mite, *Metatetranychus citri* (McG.). Areas near dusty roads exhibit this effect most frequently. Unpublished field data show that California red scale populations can be strikingly increased by frequent light applications of road dust to citrus trees in a grove in which efficient natural enemies are present. Unpublished laboratory data show that *Aphytis ling-*

*nanensis* Comp., the principal red scale parasite, suffers considerable mortality and reduction in fecundity on scale-infested fruit dusted with road dust.

Climatic extremes were found to seriously reduce certain natural-enemy populations as well as pest populations. Such effects resulted from yearly weather cycles as well as from differences in extremes between citrus areas (DeBach, Fisher & Landi, 1955).

In some cases an efficient parasite may be rendered ineffective by some asynchronism in biology or population growth with that of its host. For instance, in coastal areas *Metaphycus helvolus* (Comp.) is very effective in control of the black scale, *Saissetia oleae* (Bern.), because in those areas the black scale presents growth stages suitable for parasitism all year round. In interior citrus areas, however, the black scale has only a single generation a year, so for about six months there are virtually no scales of a size suitable for parasitism (Smith, 1942).

The adverse effects of insecticides have come to be generally recognized and accepted, especially since the advent of DDT and other powerful organics during the past decade. Perhaps just as important as the striking or obvious upsets are the long-term changes that may result from the continued use of a complex chemical control program over a period of years. In such cases insects or mites once under natural control may gradually develop to the status of minor or major pests which supposedly lack effective natural enemies (DeBach & Bartlett, 1951; Bartlett & Ewart, 1951).

Of the adverse factors mentioned above, the first two, ants and dust, can be satisfactorily eliminated by man. The next two, adverse climatic effects and lack of synchronism between host and parasite, can be overcome in some cases by periodic colonization of parasites or other manipulation. The use of insecticides can be better integrated with biological control by changes, deletions, modification of timing, dosage, or method of application. When ants and dust have been eliminated as factors affecting the faunal complex, then the maximum degree of natural control possible in the absence of insecticidal treatment can be assessed for different citrus areas over a period of several years. Some of the conclusions reached thus far follow.

#### DEGREE OF NATURAL CONTROL

As just suggested, the degree of natural control of pest populations by entomophagous species (in the absence of treatment, dust, and ants) is affected by weather conditions. Inasmuch as such conditions vary even in different citrus areas of southern California, we might expect differences in natural control between localities. The extent of these differences has often been surprising. At the present time we have to generalize from results obtained in relatively few untreated test plots in various citrus areas over the past ten years. Average results are expressed in a qualitative manner in Table I.

It is clear (Table I) that the pest status of a given species may be strikingly different in different areas. This may be due to natural enemies, climate, or more obscure factors. The pests rated "poor", "good", or "excellent" as to degree of natural control are those against which the major control practices are usually directed. In other words, these pests have been considered to lack effective natural enemies. It is evident, however, that many of these presumed pests probably would not require treatment if natural enemies were able to work unhindered. In the San Diego County intermediate area, for instance, good to excellent natural control of all pests except citrus bud mite could be expected. Citrus bud mite is a problem only on lemons; hence orange groves in this area apparently could go without treatments for California red scale, citrus red mite, and black scale, as well as for minor potential pests. Actually, in the past few years, between 1,000 and 2,000 acres of oranges in this area have been successfully put on a no-treatment program (DeBach, 1952).

In the Orange County coastal area it is seen that good natural control of all major orange pests except purple scale can usually be expected. These results have pinpointed the direction of further research, which shows great promise toward reducing current pest control costs in this area by as much as 50 per cent.

In the interior areas of Riverside and San Bernardino counties possibilities do not appear so promising. Unsatisfactory natural control of both California red scale and black scale is indicated. These are two of the most damaging citrus pests. Again, however, direction of emphasis for further research is indicated.

TABLE I. Degree of Natural Biological Control of Major Pests in Untreated Citrus Groves in Various Southern California Areas.\*

Area	California Red Scale	Citrus Red Mite	Black Scale	Purple Scale	Yellow Scale	Citricola Scale	Mealybugs	Citrus Mite lemons
San Diego County (intermediate area)	Excellent	Good to excellent	Good to excellent	N.P.	X	X	Excellent	Poor
Orange County (coastal area)	Excellent	Good to excellent	Good to excellent	Poor	X	X	Fair to excellent	Poor
Riverside-San Bernardino counties (interior area)	Poor	Fair to excellent	Poor to good	N.P.	Good to excellent	Good to excellent	Good to excellent	N.P.
Los Angeles County (intermediate area)	Poor to good	Good to excellent	Poor to good	N.P.	Good to excellent	X	Good to excellent	Poor
Ventura County (intermediate area)	—	Fair to excellent	Fair to excellent	N.P.	Good to excellent	X	Good to excellent	Poor
Tulare County (interior area)	—	—	N.P.	N.P.	Good to excellent	Poor	X	N.P.

\*Groves untreated for from 3 to 15 years. Results are indications, not recommendations. Ratings ("poor" to "excellent" are averages varying somewhat in time or in different groves, and pests so rated are those usually receiving regular pest control (chemical) treatments.  
N.P. = Not of pest status, owing to climate or host-plant resistance.  
X = Not of pest status—reasons unknown.  
— = Not generally established or under eradication program.

The degree of natural control which could be expected in the Los Angeles County intermediate area is good for most potential pests but not quite adequate in the cases of the California red scale or the black scale. Periodic colonization of red scale parasites was found to be a feasible method of obtaining satisfactory biological control of the red scale (DeBach, Landi & White, 1955). Biological control of the black scale remains a question, unless some means of increasing the effectiveness of its enemies in this area is developed. Other potential pests did not develop in the untreated plots studied.

In the Ventura County intermediate area all test plots involving oranges exhibited generally satisfactory natural control. Citrus red mite might become a problem at times in certain groves. Citrus bud mite, *Aceria sheldoni* (Ewing), on lemons does not have adequate natural enemies. This area is fortunate in that California red scale has never become generally established, owing to the employment of careful inspection and eradication methods. Furthermore, purple scale, *Lepidosaphes beckii* (Newm.), apparently cannot exist in this climatic zone. It appears that pest control practices could be reduced in this area.

In the Tulare County interior area commercial treatment has been directed primarily against the yellow scale, *Aonidiella citrina* (Coq.), the citricola scale, *Coccus pseudomagnoliarum* (Kuw.), and the citrus thrips, *Scirtothrips citri* (Moult.). Of these, the yellow scale apparently would be controlled by parasites in the complete absence of treatment. Lack of satisfactory natural control of the citricola scale and citrus thrips necessitates definite continuance of major treatment practices. Under such treatment yellow scale parasites are retarded to the extent that yellow scale is frequently the most serious pest.

The foregoing results indicate that application of the knowledge gained could now result in considerable gain in the economy of pest control in certain California citrus districts. These results also delimit problems which have to be solved in other areas.

AUGMENTATION OF NATURAL ENEMIES

There are several possible ways of overcoming weaknesses in natural-enemy efficiency, their nature depending on the cause of the weakness.

PERIODIC COLONIZATION

If a failure in climatic adaptation is evident, as was the case with the California red scale parasite *Aphytis lingnanensis*, periodic colonization of large numbers of parasites



following unfavorable climatic periods may solve the problem. Such colonizations in the intermediate area of Los Angeles County usually effected good control which otherwise would not have occurred (DeBach, Landi & White, 1955). This application depended on extensive knowledge of the effect of meteorological factors on populations of the parasite in all citrus areas. However, in the more extreme climatic areas of San Bernardino and Riverside counties, periodic colonization of *Aphytis* usually produced unsatisfactory results. A similar procedure utilizing other natural enemies is used successfully by commercial insectaries in Ventura County in groves where black scale or mealybug populations become dangerous.

#### ARTIFICIAL INOCULATION

Another possibility of increasing the effectiveness of the black scale parasite *Metaphycus helvolus* is based upon the knowledge that this parasite can attack only certain developmental stages of the scale and that these stages are virtually completely missing during 6 months of the year in single-generation black scale areas such as those of Riverside, San Bernardino, and interior Ventura counties. Climatic conditions determine the number of generations of the scale. If young scales could be artificially inoculated on citrus trees during this "host-free" period, the parasites might bridge the gap. This method was tested and the idea proven feasible, but the parasites eliminated the "inoculated" scale somewhat before the naturally occurring scale had attained a suitable size. There are still possibilities of solving this problem (Smith & DeBach, 1953).

#### COVER CROPS

Still other possibilities of improving the environment exist in the use of cover crops in citrus groves. Natural control of the citrus red mite has been augmented in experimental plots by planting permanent pasture grasses. This method provides alternate host mites on the grass as food for general predators and also serves to modify the physical environment favorably through decreased radiation, increased humidity, and dust control (Fleschner, 1956). Another type of cover crop is being tested currently for California red scale parasite retention: English ivy is being grown as a ground cover to serve as an alternate host of the California red scale and the oleander scale, *Aspidiotus hederae* (Vallot). Both of these scales are attacked by *Aphytis lingnanensis*, the principal red scale parasite. Artificial inoculation of scales on the ground cover will provide peak host populations when needed to increase parasite populations. The cover may also serve to modify usual seasonal weather conditions which depress parasite populations.

#### SELECTIVE BREEDING

Natural-enemy efficiency may be increased by the selective breeding of strains better adapted to adverse environmental conditions. This field is as yet unexplored, but possibilities are tremendous, inasmuch as natural enemies may possibly be improved greatly by slight genetic modification. For example, *Aphytis lingnanensis*, the red scale parasite, needs only a greater tolerance to slightly lower temperatures to make it generally effective. Selective breeding toward this end is being carried on.

#### INTEGRATION OF CHEMICAL AND BIOLOGICAL CONTROL

Cessation of all insecticidal treatment, as shown in Table I, would result in natural control of certain pests now receiving regular annual insecticidal treatments. In fact, in one or two areas, complete biological control might usually be obtained. However, in most citrus areas one or more pests which lack adequate natural enemies would still require treatment. The problem is to control these latter pests satisfactorily with insecticides without producing population oscillations in the other members of the faunal complex. Two of the most promising possibilities for integration of the methods are discussed below.

##### 1. Chemical Control of Purple Scale Integrated with Natural Control of Other Pests

In the Orange County coastal area, untreated test plots nearly always exhibited satisfactory natural control of all pests on oranges except purple scale (Table I). Purple scale can seriously damage orange trees and fruit when populations are dense. One parasite in particular, *Aphytis lepidosaphes* Comp., imported in 1948 from China, retards purple scale increase where established, but cannot yet, and perhaps never can, be counted upon

for consistent effective control. Tests were therefore designed to conserve populations of this parasite and other natural enemies while preventing, through chemical treatment, the increase of purple scale to damaging proportions.

Ecologically speaking, there are various means of utilizing insecticides to minimize adverse effects on entomophagous species. One of these is to treat alternately certain parts of a grove or of each tree, thus leaving untreated reservoirs of natural enemies. The method chosen depends upon the biology and ecology of the faunal complex involved. Long-term toxic residues must be avoided or natural enemies dispersing from the reservoirs will contact them and be eliminated. For this reason oil spray was chosen for purple scale treatment, and three alternate pairs of rows were sprayed at successive 6-month intervals on a progressive basis. Any given pair of rows would receive spray applications each 18 months. Thus only one third of the grove is treated at any given time, and the other two thirds continually acts as a preserve for beneficial insects. The requisite for success in this method is that the treatment give control long enough (at least 18 months) that alternation of treated and untreated rows or areas can be made at satisfactorily separated intervals, and still at times when effective results will be obtained. Either spring or fall treatment is satisfactory for purple scale control. After five years on such a program (1950–55), test orange groves in Orange County were exceptionally clean of all pests, and only one type of insecticide was used instead of the usual two or more. Furthermore, the amount of oil spray applied annually was reduced by one third.

These results were so successful that they led to new tests involving alternate oil spraying of pairs of rows on a 12-month basis. Thus only half the orange grove is treated annually, and any given pair of rows goes 24 months between treatments. There is reason to think that this modification will succeed. If it does, over-all pest control efficiency in this area should be improved, and cost of control should be cut in half.

Another approach to the same problem is to apply oil spray once a year to the north halves of all the trees in a grove. This procedure is based on the knowledge that about 90 per cent of the purple scales occur on the north half of the tree because the micro-climate is unsuitable on the south half. If successful, this program could also cut pest control by 50 per cent.

## 2. *Chemical Control of Citrus Bud Mite Integrated with Natural Control of Other Pests*

According to the results shown in Table I, the citrus bud mite is the only lemon pest in the San Diego County intermediate area requiring annual treatment. Since treatment for bud mite is necessary once or twice a year, a program of strip treatment, as with purple scale, is impracticable. A "selective-type" chemical is therefore necessary. With this knowledge in mind, materials have been tested in cooperation with L. R. Jeppson of this station for bud mite control and their effects on other pests and their natural enemies measured during the past three years. Both Aramite and Chlorobenzilate have been found to produce little if any upset in biological control and to give good control of the bud mite; hence a minimized pest control program can be recommended for lemons in this area.

## ECOLOGICAL INFORMATION AND CHEMICAL CONTROL

This paper has stressed the biological control approach to the utilization of ecological information, not only because the author is in biological control work, but also because application of ecological information toward the improvement of insecticidal control is somewhat more limited. Detailed knowledge of the biology and ecology of pests can, however, lead to distinct improvements in chemical control.

Integration of chemical and biological control has already been mentioned but should be considered essential to any chemical control program. If a certain insecticide is necessary but causes undesirable upsets in other pest populations, studies on modification of timing, dosage, or frequency of application may provide information lessening the ill effects.

The control of the cottony-cushion scale by the vedalia beetle in California is the classical example of biological control. The widespread use of DDT on citrus in the San Joaquin Valley of California just after the war virtually eliminated the predatory vedalia and resulted in a general increase of the cottony-cushion scale to the point of killing trees in some groves. As a result of studies of cottony-cushion scale and vedalia populations in relation to timing, dosage, and frequency of DDT application, Ewart & DeBach (1947)

were able to recommend modification of the spray program which still gave control of citricola scale and citrus thrips but did not cause outbreaks of the cottony-cushion scale.

Another recent organic chemical, parathion, caused serious increases in populations of the soft (brown) scale, *Coccus hesperidum* L. Bio-ecological studies by Bartlett & Ewart (1951) resulted in recommendations for modifications in treatment practices which lessened recurrence of such upsets. Artificial recolonization of parasites was also suggested after toxic residues were dissipated.

Correlation of pest population fluctuations with climate, and to a lesser extent with natural enemies, enabled Jeppson, Jesser & Complin (1953) to make definite improvements in recommendations for insecticidal control of the citrus red mite on oranges. They found that population peaks of the mite occurred in spring and fall and that treatments "applied in March and September, resulted in adequate control of this mite for the entire year. Applications made at other times of the year resulted in reduced effectiveness, as compared with that of March and September treatments."

Subsequent work by Jeppson & Fleschner (1956) on the same citrus red mite on lemons showed that seasons influence mite populations directly by causing mortality, and indirectly by determining host plant growth cycles. This influence was different in different areas. Three climatic areas were recognized: (1) coastal, (2) intermediate, and (3) interior valley areas. Low humidity exerted a major effect in reducing mite populations. As a result of these studies, different treatment timing was indicated for the different climatic zones because of differences in population cycles.

### INTEGRATED CONTROL

Practical application by the growers of the knowledge gained by ecological study is sometimes discouragingly slow. The problem is difficult inasmuch as many growers lack the entomological background to make intelligent decisions with respect to changing long-established pest control programs.

In fact, the complexities of an insecticidal pest control program, alone, on citrus are such that very few growers have enough background to obtain *optimum* results. If additional complications are introduced, such as whether or not a given pest will be controlled by natural enemies in a certain season or which insecticide will be least injurious to beneficial insects, then very few persons, even entomologists, will be capable of always making intelligent decisions. This is merely because the knowledge necessary is so detailed and specialized that few individuals are in a position to acquire it. The solution to the problem appears to lie in what has been termed "supervised pest control." I prefer the use of "integrated pest control," which implies an intelligent combination of all phases of pest control (chemical, biological, cultural, etc.) into a unified complete program.

Inasmuch as a specialist highly trained in chemical and biological control is necessary for the successful development of an integrated pest control program, it follows for economic reasons that he must supervise large acreages and have full responsibility for decisions relating to pest control. This means that pest control districts or cooperatives would have to be formed. The forerunners of such a system have been functioning rather successfully in citrus pest control in Ventura County, California, for many years. They maintain insectaries for the production of entomophagous insects, as well as establishments handling the usual spray and dust equipment. Their trained entomologists evaluate given pest situations with respect to natural enemies before deciding upon the need for insecticidal treatment. Although their programs may be improved with added knowledge, their treatment costs are among the lowest in California citrus production.

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# Aphid Transmission of Plant Viruses

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## ABSTRACT

*Aphid vector-virus relationships perhaps can be considered as forming a continuum ranging in conceptual complexity from mechanical transmission, with transmissible virus being associated with stylet tips, to that of transmissible virus being internally translocated among various body tissues prior to injection via salivary secretions. In general, the relationships are grouped into three categories—the persistent, the semipersistent, and the nonpersistent aphid-borne viruses.*

*Although it is possible to describe the how of aphid-borne virus transmission with a fair degree of detail, it is not yet possible to devise, except within broadest generalities, control measures. It seems next to impossible to eliminate aphids from any general crop area, and therefore the task of control rests on either preventing aphids from feeding on the crop to be protected, or preventing aphids which do feed from being infective. In cases where the degree of vector and/or host plant specificity are high, such an approach is at times practical; but in cases where vector and/or host plant specificity are low in degree, it appears to be impractical.*

*The results of recent researches have indicated some additional lines of attack. Modification of epidermal tissues of plants to a degree which would prevent an aphid from doing anything but testing these tissues for acceptability would stop spread of the semi- or the persistent viruses. With the nonpersistent viruses, a possible control would be modification of plant epidermal tissues so as to make these tissues incompatible with the virus and the introducing agent. In either case it is possible that applied chemicals, while not necessarily toxic to aphids as such, might prevent plant virus spread.*

Aphids are one of the major groups of arthropod vectors of plant viruses. The schematic representation in Fig. 1 is one way to summarize the position occupied by these insects in the vector array as it is now known.

Operationally, the apparent continuum of aphid vector-virus relationships can be split into many groups, but with available data three divisions are perhaps useful: the nonpersistent, the semipersistent, and the persistent. Categorization on this level is hazardous, since the differences used to contrast among group relationships are of degree rather than kind. Some of the more pertinent characteristics of the three groups are illustrated in Table I.

Perhaps the resemblances and differences among these three types of virus-vector relationships can be illustrated succinctly by discussing three fundamentals of transmission, viz., acquisition, inoculation, and retention.

**ACQUISITION.** Nonpersistent viruses characteristically can be acquired in a few seconds of feeding on diseased tissue. A theoretical curve which might be used to describe the acquisition of nonpersistent viruses by an aphid is in the nature of a second degree polynomial, i.e. some portion of a parabola (Sylvester, 1956). Between 5 and 10 seconds after assuming the attitude of penetration, the insects begin to acquire virus, with maximum efficiency being reached after 15 to 25 seconds have elapsed. If the feeding period is prolonged, loss of infectivity occurs. Data such as these, as well as far more convincing evidence from the laboratories in Canada (Bradley, 1954; Bradley and Ganong, 1955) and England (Bawden, Hamlyn, and Watson, 1954) indicate that aphids are acquiring such viruses from epidermal tissues, and as penetration is prolonged, aphids enter tissues which are relatively poor in virus content.

Data obtained from experiments with a semipersistent virus such as beet yellows indicates that the same type of curve could be fitted. However, the most obvious difference is in the time scale. The nonpersistent viruses are acquired in seconds, while the semipersistent virus is acquired in a period of hours. Such data as are available indicate that as the aphid feeds on a beet yellows source for a period of hours, there is a gradual rise in virus acquisition until a maximum is reached between 12 and 24 hours. Extension of the curve by extrapolation beyond experimental points is hazardous, and whether or not prolonged feeding decreases infectivity is not known for certain, but it seems probable that it does from

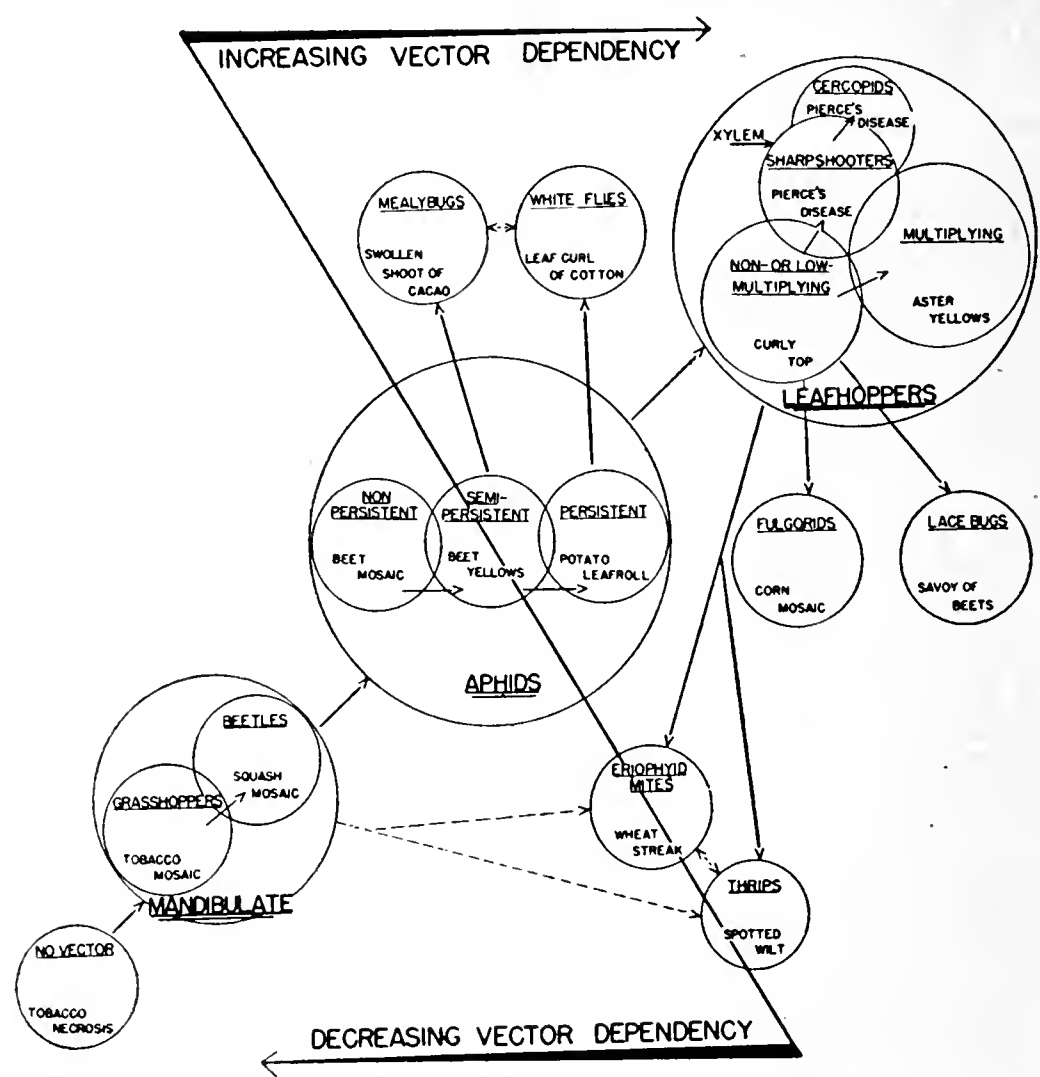


Fig. 1. Hypothetical diagram illustrating relationships of the various groups of arthropod vectors of plant viruses.

TABLE I—Operational Classification of the Vector-Virus Relationships found Among Aphids\*.

Test	Aphid-borne plant virus		
	Nonpersistent	Semipersistent	Persistent
Juice inoculation	+	±	—
Tissue of acquisition	epidermal	mesophyll-phloem	mesophyll-phloem
Preliminary fasting	+	0	0
Prolonged access time	—	+	+
Latent period	none	none	variable
Retention (feeding)	minutes to hours	hours to days	days to life
Retention (fasting)	> feeding	< feeding	= feeding
Retention (ecdysis)	—(?)	+(?)	±
Vector specificity	generally low	medium	medium to high

\*The symbols are used in the following context:  
+, (possible, or increased); —, (not normally possible, or decreased); 0, (no effect); >, (more than); <, (less than); ?, (theoretical, not confirmed experimentally).

experimental results both in England (Watson, 1946) and the United States (Sylvester, 1956).

In the case of persistent viruses, a hypothetical curve would not be parabolic in nature, but exponential, indicating that acquisition of virus increases over a period of hours until it reaches a maximum, after which time further feeding does not result in infectivity decline.

INOCULATION. Theoretical curves illustrating the manner in which time and the probability of inoculation are associated, in the case of the nonpersistent, the semipersistent,

and the persistent viruses, are all similar, with variations in the time scales. With the non-persistent viruses, the times are measured in seconds, while in the case of the semipersistent and the persistent viruses, minutes and hours are involved.

In general, it appears that the process of inoculation is not so rigidly dependent upon certain tissues as is acquisition. Perhaps this can be interpreted as indicating that virus, when once introduced, has the potential of spreading while acquisition is limited to that virus encountered during penetration.

**RETENTION.** Nonpersistent viruses, rapidly acquired and rapidly inoculated, are also rapidly lost by aphids. The period of retention is dependent upon treatment of the vectors, whether fed or fasted after acquisition, as well as upon the temperature. For practical purposes, nonpersistent viruses are not retained by feeding vectors for more than a few minutes. Examples of this phenomena have been published in which the data have been reduced to exponential curves (Smith and Lea, 1946; Sylvester, 1956).

The same type of curve can be developed with a semipersistent virus such as beet yellows, but again the difference is in the time scale. In the case of beet yellows, the half-life is approximately 8 hours, instead of a few minutes (Sylvester, 1956).

In retention of persistent viruses, the rate of loss of infectivity is much slower, and with some, such as potato leafroll (MacCarthy, 1954), decline in infectivity is scarcely measurable over the life of the aphids, while in others, such as pea-enation mosaic (Simons, 1954), the half-life probably would be calculable in terms of days.

Of interest also is the difference in the half-life of the semipersistent virus under conditions of post-acquisition fasting. With beet yellows, infectivity is lost more rapidly when the insects are fasting than when they are feeding (Watson, 1946; Sylvester, 1956). This is in contrast to the results obtained with nonpersistent viruses. With the persistent viruses, the point is difficult to check experimentally, but with those tested, a 24-hour period of fasting did not affect infectivity (Sylvester, 1950; Anderson, 1951; Simons, 1954).

One other point might be mentioned briefly at this stage, *viz.*, the latent period. In aphid vector-virus relationships, a latent period, defined as the time required to develop maximum infectivity, has only been demonstrated with persistent viruses (Smith, 1931; Osborn, 1935; Anderson, 1951; MacCarthy, 1954; Simons, 1954). It is obvious that none exists in the case of nonpersistent viruses, and thus it is only in the semipersistent viruses that the presence or absence of a latent period might be of significance. Experimental evidence to date indicates that latent periods are not found in the semipersistent virus (Watson, 1946; Sylvester, 1956).

It is hoped that this brief survey of the aphid vector-virus relationships has helped to illustrate one point, *viz.*, that the terms nonpersistent, semipersistent, and persistent, apply to areas of a continuum of vector-virus relationships which appear to exist, and although we may not be able to define precisely the limits of the groups, the classification is conceptually useful in practical discourse.

More fundamentally, perhaps, the aphid-borne viruses appear to be transmitted by two distinct processes, *viz.*, mechanical (vector direct) and non-mechanical (internal transmission, vector indirect, biological). The majority of aphid-borne plant viruses seem to be mechanically transmitted as contaminants at the tips of the stylets (Bradley and Ganong, 1955), with a minority being non-mechanically carried by a process in which the viruses presumably are ingested, absorbed into the haemocoel and haemolymph, and subsequently ejected with the salivary secretions.

A crucial experiment to establish lack of mechanical transmission is one which demonstrates infectivity to be retained through ecdysis. This assumes that molting renews the external and internal surfaces of the stylets and that no recontamination occurs during the process. Negative results, however, are indecisive, since the possibility of non-mechanical transmission is not denied. Furthermore, the two transmission processes are not necessarily mutually exclusive, for the possibility exists for both types of transmission to occur in the case of some specific vector-virus combination.

Mechanical transmission seems to be restricted chiefly to the nonpersistent viruses, viruses which appear to be acquired from and inoculated into epidermal cells. The other two groups, the semipersistent and the persistent, are conceived as non-mechanically transmitted viruses, with acquisition and inoculation being associated with mesophyll and/or phloem tissue.

Regardless of the particular scheme of classification which may be used to characterize vector-virus relationships, there is one phenomenon which is independent of classification, is basic to the understanding of vector-virus relationships, and yet which to date has eluded efforts to demonstrate its cause. This is the phenomenon of vector specificity.

Vector specificity exists in varying degrees of effectiveness ranging from complete exclusion of a species as an active vector to differences in relative efficiency of transmission. Vector specificity may be a result of virus inhibition or inactivation, if in the concept of virus inhibition or inactivation, influences of insect secretions on the susceptibility of the inoculated host cell can be included. Although the underlying principles may be similar, regardless of the type of transmission, mechanical or otherwise, the question of vector specificity is more simply posed by consideration of the results obtainable in the transmission of nonpersistent viruses, viruses which are thought to be mechanically transmitted by aphids, and which are uncomplicated by such consideration as internal inactivation, differential absorption and translocation, and the like.

Mechanical transmission implies that aphid stylets are similar to micro-needles, and that if they should enter a virus source in a favorable manner and location, they will acquire a limited amount of virus. Any subsequent penetration of a healthy plant will have a certain probability of successful inoculation. This concept is not entirely supported by experimental fact, for certain viruses, e.g., tobacco mosaic and potato X viruses, which are highly contagious and readily needle-transmitted in the laboratory, are not aphid-transmissible under usual experimental conditions. The assumption that these viruses do not occur in epidermal layers of the plant, but rather in the mesophyll, still does not explain the lack of aphid transmission. But it is not necessary to take such an extreme example, for there is ample evidence of differential transmission by various aphid species within the group of the aphid-borne nonpersistent viruses. Changing the specific virus can reverse the relative vector efficiency of two species of aphids. Also the difference in relative efficiency of two aphid species transmitting one virus from a common host can be nullified by changing the species of plant inoculated. In these cases assumptions regarding differences in virus-plant tissue relationships would not appear to be tenable. Other supportive evidence suggesting the possibility of virus inhibition or inactivation by insects could be cited, but perhaps this is sufficient for our purposes.

It has been proposed that successful inoculation of nonpersistent viruses by aphids depends to a large extent upon a compatible relationship between the cell inoculated, the virus being inoculated, and the inoculating medium (Sylvester, 1954). Whether or not saliva is introduced into every cell penetrated is open to question, but it would seem likely that when it is, it contains diffusible elements that are introduced during penetration. That the saliva is not inert appears probable from the differential effects of toxic species of aphids when on various host plants. Sometimes the effects are local, sometimes more systemic in action, with toxic species not appearing toxic on every host. Thus it is reasonable perhaps to hypothecate that salivary constituents have some influence on the physiological activities of cells penetrated, and that if these cells are also inoculated with virus, that the viral acceptability of the cells can be altered by the insect's secretions.

At the present time, it appears to be of the utmost fundamental importance to the understanding of vector-virus relationships, to ascertain whether or not such inhibitors and/or inactivators exist. As far as I am aware, we have no information as to the type of substances these proposed insect inhibitors might be, and one of the great needs of the present time is work by qualified persons on the isolation and identification of these substances.

Although the question of vector specificity and virus inhibition by insects is occupying the thoughts of many vector-virus workers today, and is a question to which I shall return later, there are many other problems to be solved concerning vector-virus relationships, as is true in any other field of investigation. Perhaps of more immediate concern is how can entomologists contribute to the solution of the existing problems in the field of aphid-borne virus diseases. Entomological aid is especially needed in the elucidation of the factors involved in the epidemiology of plant virus diseases.

Initially, a clearer picture of the biology and habits of aphids is needed, coupled with a modern, realistic appraisal of the taxonomy of the group, particularly at the generic and intra-specific levels. More needs to be known of the feeding habits and development and



dispersal of aphid populations. Is dispersal merely a result of population density, or does the growth cycle of the host plant influence this? Valuable contributions to the answers of such questions have been coming from England and the Continent.

What succession of hosts is needed by aphid vector species, both in the areas of holocyclic and anholocyclic development? What are the preferred hosts, or does this vary with the season of the year and the geographical location? Can aphid populations be predicted, if so, can cultural practices be modified to lessen their development?

More knowledge is needed on the question of how aphids actually select their host plants. Is the search a random process, or is there some obscure directive influence? Current evidence suggests both. Under what conditions will a host be acceptable, and can acceptability be artificially increased or decreased? Do aphids taste the host plants to determine the initial selection of a host, if so, where is the sensory area responsible for this?

It would be helpful to have knowledge concerning the nutritional requirements of aphids, as individuals and as populations, as well as more information on their physiology. Is it possible to rear aphids on artificial media? Attempts to do this to date have generally met with failure.

What are the functions of the salivary sheath, and what does it do to the plant tissues? Is it merely a mechanical extension of the rostrum into the plant tissues, or does it have subsidiary functions? Why are certain species of aphids capable of transmitting many plant viruses, of all types, persistent, semipersistent, and nonpersistent? Is it because they are the most frequently tried because of their proven performance, are easily cultured in the laboratory, or are there other reasons? What physiological restrictions determine that some aphid species have but one host, others only a few, while still others have many?

The list of questions could go on, but perhaps these few will point up the need for information, a need that would well deserve the attention of the ecologist, the taxonomist, the morphologist, and the physiologist, as well as the general entomologist.

To return again to the general topic of viral inhibitors or inactivators within the aphids, one of the most intriguing possible lines of attack, and one which might not only answer some questions concerned with the problem of vector specificity, but also might help in the problem of field control of virus spread, is that concerned with the modification of the host plants.

For example, in a typically nonpersistent virus, one which is mechanically borne on the tips of the stylets, the transmission cycle in nature is envisioned as being something like the following: virus source plants exist in the field. Potential aphid vectors may or may not breed on these particular virus source plants, either individually or collectively, but in the random search for new hosts, various aphids sample the epidermal layers of these infected plants and thereby acquire a limited charge of virus. These particular viruses appear to reach high concentrations in the epidermal layers of their host plants. Thus it is during the random sampling of epidermal tissues by aphids in search of acceptable host plant, that infectivity is accidentally acquired. Following acquisition, the aphids may or may not move to another plant, but those that do continue the search, probing the epidermis of potential hosts, and thus inoculation is accomplished. Under such conditions, spread is considerably aided by the concentration of susceptible hosts in agricultural areas.

Success of inoculation is probably governed by many factors, but if one of these is the hypothesized compatibility between the cell inoculated, the virus involved, and the inoculating medium (the salivary secretions of the aphid species involved), it then seems possible that the exidermal cells of susceptible hosts could be slightly modified so that the probabilities associated with successful inoculation would be drastically reduced. Reduction of viral infectivity by artificial means is not new, for it has been repeatedly demonstrated in connection with juice inoculable viruses and such physical agents as ultra-violet light (Bradley, 1954; Bawden, Hamlyn, and Watson, 1954), as well as with a long list of chemical agents of varying complexity (Gupta and Price, 1952). To date such experiments have indicated that if the effects are due to reduction of host plant susceptibility, it is temporary, and the mode of action is not clearly defined. In any case, little, if anything, is known of the action of such agents under conditions of insect inoculation. Perhaps epidermal modification could be accomplished genetically as well as chemically. In any event, the possibilities are open to investigation.

One of the basic assumptions to such an approach is whether or not epidermal tissues are the only tissues utilized by aphids in inoculating plants with this group of viruses. Evidence on this point to date is not conclusive, but probably soon will be forthcoming.

So much for the nonpersistent aphid-borne viruses. Of equal importance and concern are the groups of semipersistent and persistent viruses. In general it appears that with these viruses the process of acquisition and inoculation is relatively slow, indicating that the aphids must penetrate rather deeply into plant tissues either to acquire or to inoculate them. Usually the process of acquisition is slower than that of inoculation, perhaps meaning that the areas of active virus concentration are more localized than are the areas from which infection can be initiated. Confronted with this type of relationship, what can be done?

First of all, for an aphid to penetrate deeply into plant tissues, it might be logical to assume that it has been given some sensory assurance that it is worthwhile. If determination as to whether or not an aphid is going to penetrate for a long or short period of time is governed by the sampling of epidermal tissues, it would then appear that there exists the distinct possibility of modifying these tissues in such a way as to discourage deep penetration by aphids. Again this might be done genetically, and perhaps much of the program of selection of plant resistance to aphid attack is dependent upon this principle. The other avenue open is chemical, for it might be possible and feasible to treat plants in such a manner as to render the epidermal tissues unacceptable to aphids.

Thus there are two possible lines of attack, which to date have been explored, if at all, in a minor way by entomologists. Normally the economic entomologist is interested in killing insects, and perhaps by rigorously adhering to the principle of mortality in the presence of toxicants, agents which might be effective in the control of aphid populations have been overlooked or discarded. Perhaps it would be useful to think of the possibility of killing aphids less directly or immediately, but just as certainly, through modification of their host plant in such a way as to make them unacceptable to colonization.

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## DISCUSSION

M. F. DAY. How do you think semipersistent viruses are transmitted?

E. S. SYLVESTER. By essentially the same mechanism as the persistent viruses.

R. C. DICKSON. Is not the problem of vector specificity related to vector efficiency?

E. S. SYLVESTER. I believe that they are degrees of the same phenomenon.

D. HILLE RIS LAMBERS. Are there any data available on the prolongation of the half-life of nonpersistent viruses by very low temperatures? If the answer is positive, this would materially affect our views on long distance spread of nonpersistent viruses.

E. S. SYLVESTER. Limited data indicate that lowering the temperature increases the half-life of nonpersistent viruses in fasted aphids.

# Transmission of Virus by Leafhoppers

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## ABSTRACT

Experience with the electron microscope and serology of three leafhopper-borne viruses emphasizes the value of these techniques for ascertaining the natural relationships of such viruses.

It has been shown that viruses distinctly different in morphology and serology may be transmitted by the same leafhopper.

It is suggested that cross-protection between viruses in leafhoppers may be indicative not only of relationship but of virus multiplication in the vector.

In general individual plant viruses are apparently transmitted only by vectors in one of the major arthropod taxa. There is need for systematic testing of this group specificity of transmission.

Recently described vectorless forms of plant viruses that are normally arthropod-transmitted are discussed. They serve to emphasize transmissibility as a positive hereditary attribute subject to mutation.

Some 12 years ago I proposed the genus *Aureogenus* to accommodate four viruses (1, N.Y. potato yellow-dwarf virus, 2, N.J. potato yellow-dwarf virus, 3, wound-tumor virus and 4, clover club-leaf virus) transmitted by species of agallian leafhoppers (Black, 1944). Each virus exhibited different vector relationships. At the time I was much impressed by the fact that all of the vectors were agallian leafhoppers, and was persuaded that this and the symptoms induced in plants indicated relationship among the viruses. In the case of the first two viruses it was possible to obtain additional evidence of relationship by means of cross-protection tests in *Nicotiana rustica* L. and it seemed probable then that all 4 viruses belonged together in a single genus.

As a result of the development of novel methods of virus purification (Brakke, 1953, 1955) and their application to the successful purification of the first three of these viruses, it was possible to study their morphology and serology so that we now know much more about their relationships. Our present knowledge clearly indicates that my earlier inferences based on vector relationships and symptoms were partly right and partly wrong.<sup>1</sup> Under the electron microscope the first two viruses may appear, in what we consider the best preparations thus far made, as roughly spherical particles about 135 m $\mu$  across (Black, Mosley and Wyckoff, 1948; Brakke, Black and Wyckoff, 1951; Brakke, Vatter and Black, 1954). They may also appear very much flattened or as short rods. At present unknown differences in the preparation of these viruses for observation with the electron microscope appear to cause drastic variations in their shape. The third virus is morphologically different appearing as a polyhedron about 75 m $\mu$  across (Brakke, Vatter and Black, 1954; Black, 1955). The first two viruses have antigens in common but also have antigens that are distinct (Wolcyrz and Black, 1956) and are not serologically related to the third virus (Black and Brakke, 1954).

The clear cut nature of this morphological and serological evidence for relationship or lack of it indicates the desirability of obtaining this sort of data for other leafhopper-borne viruses. It also permits us the opportunity to reassess the criteria that were employed earlier in attempting to classify these viruses. Actually, a more critical appraisal of the symptoms produced by the three viruses emphasizes the similarity of those caused by the two potato yellow-dwarf viruses and their dissimilarity from those caused by the wound-tumor virus. The conclusion from cross-protection tests with the first two viruses is sustained. But the inference of relationship that was drawn from the fact that all vectors were agallian has proven equivocal. There is, of course, only a small chance that any two viruses picked at random would prove to be closely related, so that the relatedness of viruses 1 and 2 may indicate that the relationship of their vectors did have some significance in indicating

<sup>1</sup>Our knowledge of the relationships of the fourth virus, the clover club-leaf virus, is little better today than it was at the time it was placed in the genus *Aureogenus*. At present there seems to be insufficient evidence for considering it closely related to any of the first three viruses and it will not be discussed further in this paper.

the relationship of the viruses. However, the lack of relationship between these two viruses and the third virus, even though efficient vectors of 1 and 2 are related, and of 2 and 3 are, in some instances, identical (Black, 1944), demonstrates that this criterion is an uncertain one.

Kunkel (1955) has recently shown that certain leafhopper-borne viruses, namely, the type variety of aster-yellows virus and the celery-yellows strain, cross-protect in the vector. Previous evidence of the relatedness of these viruses was based solely on the symptoms they produced and on a common vector. Kunkel's work provides us with a new and valuable criterion for testing relationships of viruses that have a common leafhopper vector. It may be, however, that this criterion is applicable only in those cases where the virus multiplies in the vector. Such multiplication has been demonstrated for the type variety of aster-yellows virus and presumably holds for the celery-yellows strain. The lack of cross-protection, in the vector, between various strains of curly-top virus (Giddings, 1950) may be related to absence of multiplication of that virus in its leafhopper carrier (Freitag, 1936; Bennett and Wallace, 1938). Cross-protection in the vector may prove to be not only an indicator of relationship of viruses but of virus multiplication in the vector.

Do the vector relationships of plant viruses give us any information about the relationships of the viruses themselves? If one consults lists of vectors (Heinze, 1951; Day and Bennetts, 1954) one will find, below the name of a virus, many or all the insects that have been reported to transmit that particular virus. This information is most useful but, even when the author indicates that some reports are of doubtful validity, I think it tends to obscure what may be a very important fact, namely, that, in general, the vectors of a plant virus belong to a specific group. With a few possible exceptions, there are no well authenticated cases of a plant virus being transmitted by more than one arthropod belonging to any two of the following groups: aphids, leafhoppers, white flies, thrips, tingids, mealy bugs, or mites. The generalization would be more obvious in lists including only well established vectors. Undoubtedly there are many reports of vectors based on insufficient evidence, mistaken diagnosis of disease, contaminations, unsuspected virus mixtures, mistaken identity of the transmitting agent and other errors.

I am aware that there are some plant viruses for which there is evidence contrary to the foregoing generalization. Many years ago evidence was presented indicating that the virus of potato spindle-tuber is transmissible by aphids (Schultz and Folsom, 1925) and by grasshoppers, four species of beetles and the tarnished plant bug (Goss, 1931). Tobacco mosaic virus has been reported to have been transmitted by a variety of insects: aphids, grasshoppers, thrips, mealy bugs, leafhoppers and others (Heinze 1951; Day and Bennetts, 1954). For some of these vectors the evidence is substantial. Among vectors reported for cucumber mosaic virus may be mentioned aphids and cucumber beetles. Doolittle and Walker (1925) presented considerable experimental evidence of the ability of these insects to transmit cucumber mosaic virus. However, recently Freitag (1952, 1956) in a study of viruses occurring on cucurbits in California has found that these viruses, which included strains of cucumber mosaic virus, were transmitted by beetles or by aphids but not by both. Freitag's work has raised doubts about the transmission of cucumber mosaic virus, *Marmor cucumeris* H., by beetles and makes it desirable to repeat Doolittle and Walker's tests as closely as possible. Many other reports running counter to the above generalization regarding the specificity of transmission by groups of insects need to be re-investigated.

Entomologists could greatly advance our knowledge of this whole problem of the range of vectors that will transmit particular plant viruses. Frequently plant pathologists, confronted with a plant virus disease, have been impelled to hunt for a vector. Many have succeeded in finding an important one and have then proceeded to study other aspects of their disease problem. Sometimes they have studied transmission itself but usually they have confined such study to the vector they have been so glad to obtain. Often the only data indicating the failure of other insects, in other major groups, to transmit the virus is that accumulated during the original search for a vector. This is frequently considerable and the bulk of evidence over the whole field which supports the generalization on the group specificity of vectors is very impressive. Day's recent table (Day, 1955) listing the number of viruses reported to be transmitted by certain major insect taxa may contain some viruses listed under more than one taxon. However, it may serve to give an approximate idea of a minimal number (85) of viruses to which this group specificity seems to apply. This



specificity has not been studied systematically and entomologists are well qualified to contribute to such a systematic study and either establish such a generalization firmly or expose its fallacies.

Even if there are some plant viruses that are transmitted indiscriminately by more than one major group of insects, the specificity of plant virus transmission by one major insect taxon appears to have validity for a wide range of plant viruses. There is much evidence already to sustain such a view. It follows that most plant viruses must have different hereditary characters corresponding to the different vector groups. This heredity is probably controlled by genes or gene-like factors within each virus and these are probably subject to mutation.

Have we recognized sufficiently that transmissibility of a virus by particular vectors is a positive attribute of the virus? Let us contrast this attribute for a moment with the fact that all plant viruses are unable to be transmitted by a great host of insects. The simple morphological forms thus far disclosed for plant viruses may mislead us into taking their modes of transmission for granted or into delaying scientific investigation of their mechanisms of transmission. In them, the electron microscope has not revealed anything corresponding to the highly specialized tail mechanism of the phage with its ultra-fine tendrils which presumably increase the probability of contact with the host bacterium and may have other functions in attachment and penetration (Williams and Fraser, 1956). Actually the surface chemistry of plant virus particles may be highly complex and importantly involved in the specificities of transmission. The constantly increasing number of serologically distinct groups of plant viruses provides an indication of abundant diversity in the chemical structure of the plant virus surfaces.

The occurrence of vectorless strains of certain plant viruses that are normally insect-transmitted also has a bearing on this problem. In 1953, 1 isolate of the N.Y. potato yellow-dwarf virus and 2 isolates of the N.J. potato yellow-dwarf virus were reported to have lost their ability to be transmitted by their vectors after being maintained for many years in *N. rustica* without any transmission by insects (Black, 1953). At that time the only knowledge about the derivation of these vectorless forms was the history of the isolates. Since then absorbed antisera specific for the N.Y. and N.J. potato yellow-dwarf viruses have been obtained (Wolcyrz and Black, 1956). The three viruses and their subviruses react with absorbed antiserum specific for N.Y. virus but not with that specific for N.J. virus, (Wolcyrz and Black, 1957), strongly indicating that all three were derived from the N.Y. virus. This means that the evolution of only one isolate of the potato yellow-dwarf virus to a vectorless form has been demonstrated.

How does the vectorless virus differ from the vectorial? It certainly differs from the latter in some hereditary character and consequently its origin is probably due to one or more mutations in the virus genes or gene-like factors. There is some evidence that its vectorless character is not due to a lowering of the virus concentration in the host. Extracts from plants used as a source of virus for vectors showed that the vectorless form produced more primary lesions than the vectorial. This result, however, may be attributable to evolution of the vectorless form along another pathway, during the same period in which it became vectorless, so that it would be better adapted, than is the vectorial form, to inoculation by rubbing juice from diseased *N. rustica* onto healthy leaves of the same plant and so would give more lesions with equivalent amounts of virus. Our experience in purifying the vectorial and vectorless forms suggests that the vectorial is probably slightly more concentrated in *N. rustica*. However, there are many ways, other than changes in concentration, in which vectorless virus may be conceived to have arisen, such as, its failure to be absorbed on, to penetrate into, to multiply in or to escape from the cells of the vector. It seems certain that other vectorless forms of viruses, normally insect-transmitted, will be found in the laboratory and it seems likely that they will also be found in the field. Because it would seem probable that there is a hereditary basis for group specificity of vectors in most plant viruses, even in those that are non-persistent, it also seems probable that in all plant viruses transmitted by vectors, mutations to vectorless forms could occur. If long reproduction in plants without the intervention of the vector is a determining factor in the selection of such forms of virus they may well occur in clonally reproduced plants whether wild or cultivated. Any search for the vectors of such forms would appear to be fruitless even if the search were extensive. Their relationship to vectorial forms might be indicated

by study of their serology and other properties. However, tests of vectors related to that of the vectorial form are desirable before the vectorless nature of any virus is inferred. For example, it might have happened that the N.J. potato yellow-dwarf virus would have been discovered in plants, shown to be serologically related to N.Y. potato yellow-dwarf virus and demonstrated not to be transmitted by the principal vector of the N.Y. virus, *Aceratagallia sanguinolenta* Prov. These facts might have been interpreted to indicate it to be a vectorless form of the N.Y. virus. Instead of being vectorless, however, this virus is transmitted by another agallian leafhopper.

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## DISCUSSION

R. W. CHAMBERLAIN. How was evidence of cross-protection of related plant viruses in leafhoppers obtained? Also, is there evidence of cross-protection between related plant viruses in the plant host?

L. M. BLACK. Kunkel showed that leafhoppers that had acquired one kind of aster yellows virus could not subsequently acquire and transmit a different kind of aster yellows virus. He also demonstrated cross-protection in plants between the same two kinds of aster yellows viruses.

# Mandibulate Insects as Vectors of Plant Viruses

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## ABSTRACT

Beetles and grasshoppers are the most important vectors of plant viruses transmitted by insects with mandibulate or chewing mouthparts. Ten or more plant viruses have been shown to be transmitted by mandibulate insects, with the most significant results being obtained on the transmission of cowpea mosaic, squash mosaic, turnip yellow mosaic and tobacco mosaic viruses. None of these viruses has been conclusively demonstrated to be transmitted by both mandibulate and suctorial insects.

It has been suggested that transmission by mandibulate insects represents the most primitive type of relationship between virus and vector. This conclusion is based on the assumption that since mandibulate insects transmit several highly infectious plant viruses, such transmission occurs by mere mechanical contamination of mouthparts. However, a more intimate relationship is indicated by specificity of vectors and by recovery of active virus from blood, the extract of crushed bodies, regurgitated fluids and the feces of beetles. The retention of the virus by beetles for a period of twenty days indicates internal uptake and ejection into the plant. The mechanism of transmission of plant viruses by insects with mandibulate mouthparts is not known, but it is probably by a process of regurgitation, although transmission through salivary secretion or defecation has not been ruled out. The results suggest a high virus concentration in the body of the beetles and indicate virus movement through the intestinal wall and survival in the blood. The evidence implies a more complex relationship between beetle and virus than exists between nonpersistent viruses and their aphid vectors where active virus can be demonstrated as existing only on the tips of the stylets of the mouthparts.

## INTRODUCTION

Doolittle (1920) was the first to report that plant viruses were transmitted by mandibulate insects when he announced that cucumber mosaic virus was transmitted by two species of cucumber beetles in addition to the melon aphid, *Aphis gossypii* Glover. However, his claim that beetles are vectors of cucumber mosaic virus has not been confirmed. C. E. Smith (1924) demonstrated the bean leaf beetle, *Ceratoma trifurcata* Forst., to be the vector of cowpea mosaic virus, but his work received scant attention until Dale (1949) 25 years later reported transmission of a cowpea mosaic virus by a leaf beetle in Trinidad.

Beetles and grasshoppers are the most important vectors of plant viruses transmitted by insects with mandibulate or chewing mouthparts. The flea beetles, *Phyllotreta undulata* and *P. cruciferae*, and the mustard beetle, *Phaedon cochleariae*, and its larva transmit turnip yellow mosaic in England (Markham and Smith, 1949). The flea beetle, *Epitrix* sp., was reported to transmit egg plant mosaic (Dale, 1954). The leaf beetle, *Ceratoma ruficornis* (Oliv.), has been found to be responsible for the natural spread of cowpea mosaic (Dale, 1949). Cucumber beetles, *Acalymma trivittata* (Mann.) and *Diabrotica undecimpunctata* Mann., are considered to be the natural vectors in the field spread of squash mosaic, cucurbit ring mosaic and wild cucumber mosaic viruses in California (Freitag, 1941). Four species of beetles, *Epitrix cucumeris* (Harr.), *Systema taeniata* (Say), *Disonychia triangularis* (Say) and *Leptinotarsa decemlineata* (Say), were reported to be vectors of spindle tuber and unmottled curly dwarf viruses of potato (Goss, 1931).

Grasshoppers, *Melanoplus* spp., have been reported to be vectors of potato spindle tuber (Goss, 1931), tobacco mosaic, potato X, and tobacco ringspot (Walters, 1952), but the part if any that grasshoppers play in the natural spread of these viruses has not been determined. The transmission of tobacco mosaic virus by the differential grasshopper, *Melanoplus differentialis* (Thos.), appears to be a purely mechanical type of transmission (Walters, 1952). The turnip yellow mosaic virus has been demonstrated (Markham and Smith, 1949) to be transmitted by the long-horn grasshopper, *Leptophyes punctatissima* Bosc., and the short horn grasshopper, *Stauroderus bicolor* Charp.

Several plant viruses have been reported to be experimentally transmitted by a variety of mandibulate insects. In addition to beetles and grasshoppers, the turnip yellow mosaic

virus (Markham and Smith, 1949) is transmitted by the common earwig, *Forficula auricularia* Linn., but not by means of aphids, plant bugs or caterpillars. Although the potato spindle tuber virus is also transmitted by beetles and grasshoppers, Goss (1931) claims that two insects with suctorial mouthparts, the tarnished plant bug and green peach aphid are capable of transmitting the virus. Insects are under some conditions considered to be ineffective vectors of the virus since the spindle tuber virus is readily spread by contamination of the seed stock by mechanical means during the process of storing, handling and planting the crop.

Caterpillars of *Plusia gamma* L. have been reported to transmit tobacco mosaic virus (Sukhov and Vook, 1945).

## CHARACTERISTICS OF VIRUSES TRANSMITTED BY MANDIBULATE INSECTS

The viruses transmitted by insects with mandibulate mouthparts appear to be of a completely different type from the nonpersistent aphid-borne viruses and several of them have been found to be very suitable for more fundamental studies on the nature of the virus particle. These viruses are highly infectious, stable in plant extracts and easily transmitted by sap-inoculation. The squash mosaic virus (Takahashi, 1948) and turnip yellow mosaic virus (Markham and Smith, 1946) were among the first plant viruses to be purified, crystallized and shown to be spherical particles. These viruses have also been demonstrated to be serologically active.

The work on turnip yellow mosaic virus was one of the first to give an indication of the location of the nucleic acid component in a plant virus particle (Markham, 1953). Purified virus preparations consisted of 80 per cent nucleoprotein and 20 per cent of a nucleic acid-free protein. While nucleic acid-free protein particles were not infectious, they had the same size and shape under the electron microscope and had similar serological and electrophoretic reactions. The active nucleoprotein virus particle was considered to be a sphere made up of an outer shell of protein and a central core of nucleic acid.

Squash mosaic (Freitag, 1956), cowpea mosaic (Dale, 1949), and turnip yellow mosaic (Markham and Smith, 1949) have limited host ranges and in most instances can be transmitted to only one plant family. Squash mosaic (Middleton, 1944) and cowpea mosaic (Dale, 1949) are seed-borne in certain of their host plants.

The search for insect vectors of this group of viruses has been neglected, because they are easily sap transmitted and their properties determined without the use of their insect vectors. The mandibulate insects are not easily reared in sufficient numbers for transmission experiments and this fact has necessitated the use of field collected specimens which are less reliable for use in experimental work and add to the difficulties.

## ATTEMPTS TO TRANSMIT WITH SUCTORIAL INSECTS

Numerous attempts have been made to transmit the three most thoroughly investigated beetle-borne viruses by aphids, leafhoppers and plant bugs. The results indicate that these viruses are not transmitted by any suctorial insects. Turnip yellow mosaic virus was not transmitted in experiments with the green peach aphid or the cabbage aphid (Markham and Smith, 1949). Three species of plant bugs tested also failed to transmit the virus and since these were the only sucking insects observed among field grown turnips in sufficient numbers to be considered as vectors it was concluded that insects with suctorial mouthparts play no part in the natural spread of the virus. Cowpea mosaic was not transmitted in repeated tests with aphids using varying feeding periods and including preliminary starvation (Dale, 1953).

Squash mosaic was not transmitted by any of nine species of aphids tested (Freitag, 1956). None of 450 squash test plants became infected following aphid feeding. In addition, 5 species of leafhoppers and 2 species of plant bugs failed to infect 140 squash plants.

There is now good experimental evidence that turnip yellow mosaic, cowpea mosaic and squash mosaic viruses are transmitted by mandibulate insects, but not by insects with suctorial mouthparts. However, it is still possible that certain other viruses may yet prove to be transmitted by both mandibulate and suctorial insects. The tobacco ringspot virus, for example, has been reported to be transmitted by grasshoppers (Walters, 1952) and by



the green peach aphid to gladiolus (Smith and Brierly, 1955), but was not transmitted by aphids to tobacco and cucumber (Pound, 1949). Cucumber mosaic and cabbage mosaic are typical nonpersistent aphid-borne viruses and the claim that the former is also transmitted by cucumber beetles (Doolittle, 1920) and the latter by caterpillars (Larson and Walker, 1939) need confirmation. Present knowledge suggests that such reports claiming transmission by both mandibulate and suctorial insects be reinvestigated and substantiated before they can be accepted as established insect vector-plant virus relationships.

### INSECT VECTOR SPECIFICITY

Specificity among insect vectors of plant viruses has received considerable attention and has been regarded as an important fundamental relationship. However, the significance of insect vector specificity has not been ascertained. It has been shown that there are varying degrees of specificity and vector efficiency among the aphid and leafhopper vectors of plant viruses. This ranges from lack of ability to transmit through various degrees of efficiency to highly efficient transmission.

The two cucumber beetles demonstrated to be vectors of squash mosaic virus varied greatly in their efficiency to transmit (Freitag, 1956). The smaller of the two beetles, the western striped cucumber beetle, was the most efficient vector and transmitted the virus to 92 per cent of the plants when 10 beetles were fed per test plant and 40 per cent when a single beetle was used. The western twelve-spotted cucumber beetle infected only 40 per cent of the plants when 10 beetles were used and 8 per cent with single beetles. The results indicate a definite variation in efficiency of these two beetles to transmit the virus and provide additional evidence against the mechanical transmission hypothesis. It would appear that if the beetles transmitted the virus by mere external contamination of the mouthparts the larger beetle, the western twelve-spotted, might be the more efficient vector, or at least equally efficient, but the reverse is true. In addition it was shown in a limited number of tests that a third species occurring in California, the banded cucumber beetle, *Diabrotica balteata* Lec., apparently lacks the ability to transmit the virus to healthy squash plants. These results provide evidence for specificity among the mandibulate vectors.

### MANDIBULATE VECTOR-VIRUS RELATIONSHIPS

The following discussion will attempt to elucidate certain of the features of the insect vector-plant virus relationships of the mandibulate vectors so that comparisons can be made with plant viruses transmitted by other insects.

Turnip yellow mosaic virus has been transmitted following acquisition feedings as short as one minute (Markham and Smith, 1949) and cowpea mosaic in periods of less than five minutes (Dale, 1953). The squash mosaic virus can be acquired by the western striped cucumber beetle after a short feeding period of only five minutes and can infect healthy plants following a similar short feeding period (Freitag, 1956). Little work has been done to determine if any latent period exists in the beetles before they can transmit. Markham and Smith (1939) suggest a latent period exists in the transmission of turnip yellow mosaic virus, but offer little data in support of it. Beetles fed for five hours on infected squash plants transmitted squash mosaic virus during the following five-hour period.

Transmission experiments were conducted to determine how long infective cucumber beetles could retain the squash mosaic virus when transferred daily to healthy squash plants. The beetles infected plants as long as 20 days following their last feeding on diseased plants (Freitag, 1956). Most of the beetles infected a high percentage of the plants on which they fed the first few days, but lost the ability to infect plants rather rapidly. Individual beetles infected plants at very irregular intervals and the long intervals between infections are hard to interpret. The bean leaf beetle vector of cowpea mosaic remained infective for 14 days, much longer than the survival of virus in extracted plant sap (Dale, 1953). Turnip yellow mosaic was retained for 7 days by its beetle vectors (Markham and Smith, 1949). Beetles having longer access to infected plants transmitted more efficiently than those permitted shorter access periods.

The retention of squash mosaic virus for 20 days by the cucumber beetles under experimental conditions suggested the possibility that the virus might overwinter in the bodies of the beetles under natural conditions. In order to obtain evidence on this question

adults of the western striped cucumber beetle were collected in February on weeds not known to be susceptible to the virus. The beetles were collected in an area where no squash plants were observed at the time. The overwintering beetles were transferred to healthy seedlings in the greenhouse. Several transmissions resulted, demonstrating that the adult beetles were carrying the virus and that the virus may have overwintered within the body of the insect (Freitag, 1956). The fact that beetles retained the ability to transmit virus for a period of 20 days indicates a more intimate relationship than mere mechanical transmission of the virus by externally contaminated mouthpart surfaces as has been assumed by some in the transmission of viruses by mandibulate insects.

Several authors have reported that mandibulate insects do not possess salivary glands. Dale (1953) reports that F. J. Simmonds (personal communication) found no functional salivary glands nor oesophageal valves in beetles like *Ceratoma ruficornis*, *Phyllotreta* or *Phaedon*. However, Pradhan (1937) reports that salivary glands are present in reduced form in the family Coccinellidae of the order Coleoptera. If the latter is true it is possible that transmission of viruses by beetles may be through a process of salivary secretions. The grasshoppers have well developed salivary glands although they also regurgitate.

Markham and Smith (1949) have suggested that mandibulate insects transmit turnip yellow mosaic virus through the process of regurgitation. Work on squash mosaic transmission by beetles (Freitag, 1956) showed that when infective beetles were anesthetized with ether they would regurgitate a clear liquid of greenish amber color. This liquid was absorbed on a cotton swab on the end of a pin and inoculated on the cotyledons of squash plants. A high percentage of squash plants developed infection demonstrating that active virus was present in the regurgitated fluid.

In general, inoculation of plants with juices of crushed infective insect has failed to result in infection. Black (1939) showed that the addition of juice from a number of insects to inoculum containing viruses either reduced the infectivity or rendered the mixture non-infective. He concludes that the apparent general inhibiting action of insect juices upon the infectivity of plant viruses is one reason for general failure to successfully inoculate plants with the juice of infective insects.

In contrast, squash mosaic (Freitag, 1952) and cowpea mosaic (Dale, 1953) viruses were readily recovered from the bodies of crushed beetle vectors by inoculating healthy test plants. Squash mosaic was recovered from the crushed bodies of beetles following a 5-day feeding period on healthy plants, but not following a 12-day feeding period. Virus was also recovered from naturally infective beetles collected in melon fields.

Recovery of squash mosaic virus from the crushed bodies of beetles raised the question of the location of the virus within the body of the insect. Squash mosaic virus was recovered from the blood of infective beetles (Freitag, 1956). The hind legs of infective beetles were pulled off and light pressure applied to cause blood to ooze out through the wound. Blood was picked up on a dissecting scalpel and rubbed on a cotyledon of a squash plant. Blood was also obtained by making a shallow incision on the abdomen with dissecting scalpel. These experiments demonstrated the presence of active virus in the blood of infective beetles.

Active squash mosaic virus was demonstrated in the feces of beetles 10 days following their last feeding on infected plants (Freitag, 1956). Twenty-five beetles were collected from a squash mosaic infected plant and transferred to a sterilized test tube for a period of one hour following which they were removed and the test tube was washed with distilled water. The wash was inoculated to healthy plants and proved to be infectious.

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## DISCUSSION

L. M. BLACK. Did you test the possibility that juices from beetles inhibit infectivity when the virus is inoculated by rubbing? It would be very interesting if there was no inhibition in this case.

J. H. FREITAG. The tests you suggest have not been made.

M. A. WATSON (MRS.). If the beetles have no salivary excretion and stomach regurgitation takes its place, presumably the virus must return from the blood to the stomach in order to be returned to plants.

M. F. DAY. A comment may be made on Dr. Watson's suggestion that virus comes from the blood back into the gut, for it may well be that enough virus remains in the gut and its diverticula to account for infection by regurgitation.

M. A. WATSON (MRS.). The virus must come from somewhere as it would not remain in the stomach for 20 days.





# Transmission of Virus by Mosquitoes

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## ABSTRACT

Mosquitoes transmit viruses by two mechanisms. Viruses in the first group are exemplified by the viral encephalitides. Virus is ingested during a blood feed, multiplies in the mosquito following a latent period, and is injected into a susceptible host in the saliva. Relatively high viremia is necessary to infect a mosquito and there is evidence for a barrier to infection in the midgut. Viral multiplication can occur in many mosquito tissues, but no instance of survival of infection in the egg to the following generation has been substantiated. Injection of virus into the hemocoel can initiate virus multiplication, but it does not necessarily make a species infectious. There may thus be a barrier to virus penetration into the salivary glands. Species of mosquitoes vary in this infectability and there is evidence for marked intraspecific differences also. No deleterious effect of the virus on the mosquito has been reported.

Viruses in the second group are exemplified by the pox viruses. These are transmitted mechanically. All that have been adequately studied produce viruliferous dermal lesions and the virus responsible for infection contaminates the stylets. A method for estimating the mean number of infectious particles acquired by a mosquito during a feed on an infectious lesion has been developed, and the evidence indicates that the virus content of a lesion must approximate  $10^7$  infectious particles per gram before a mosquito is able to acquire a dose sufficient to become infectious.

The classical concept of mosquito transmission of pathogens stems from the early work on malaria. In this it could be demonstrated visually that the protozoan parasites were ingested with the blood meal, penetrated the midgut epithelium, eventually reached the salivary glands and were inoculated into a vertebrate with the saliva.

It was assumed in the early work on yellow fever, and later with other mosquito-borne viruses, that the same mechanism was involved, and subsequent work with other viral encephalitides has confirmed that this is so. The current view is that, when sufficient virus is ingested, enough survives to penetrate the midgut epithelium. Either here or in other tissues of the body the virus multiplies, and generally after a latent period of a few days, enough is present to reach the saliva. There is evidence that the midgut is a barrier to penetration, for when it is punctured the vector efficiency of poor vectors has been increased (Merrill and Ten Broeck, 1935). When certain viruses are inoculated into the hemocoel of certain species of mosquitoes, the virus may multiply, but the mosquito never acquires the ability to transmit the pathogen (McLean, 1955). It is, therefore, surmised that the salivary glands present a second barrier to virus penetration.

There is a great deal still unknown about this hypothesis of transmission, but in outline it is unquestionably correct. It is uncertain what mosquito tissues will or will not support virus multiplication, although Trager (1938) showed in tissue culture that quite a number of tissues could be involved. It is not known how many cycles of virus multiplication occur before the virus is excreted by the salivary gland. The effect of temperature on virus multiplication has been carefully examined by several authors, the most recent being Chamberlain and Sudia (1955). They present some evidence to suggest that at 70°F the cycle of Eastern Equine Encephalitis (EEE) in *Aedes triseriatus* approximates 4 days. Virtually nothing is known about the mechanism of virus multiplication in the vector or what determines the characteristic changes in virus titer with time after infection although this relationship has been found with all of the encephalitides so far studied. Nothing is known about how the viruses penetrate the cell membranes. Some attempts have been made to determine the amount of ingested virus necessary to infect the vectors and the amount inoculated by a feeding mosquito (e.g., Chamberlain *et al.*, 1954). But there is still much to be learned about even these details. The availability of insect tissue cultures would assist in solving some of these problems, and with the number of workers pursuing this field it is to be hoped that adequate tissue cultures will be available before long.

Many of the same questions, are, of course, of interest with leafhopper-borne viruses but cannot be examined until improved methods of assaying virus concentrations are available.

This transmission mechanism has come to be known as the “*biological*” mechanism of transmission. It has a number of interesting consequences. The existence of the barriers to virus penetration is reflected in marked differences between mosquitoes in their ability to acquire infection. Thus, Chamberlain and his colleagues have found that some species may become infected when the blood titer of the host is as low as  $10^2$  MLD 50, whereas other species require a titer of  $10^7$  or higher. The interesting observation has been reported for Rift Valley Fever that when the mouse blood is about  $10^8$  LD<sub>50</sub>/0.03 ml. blood the mosquito is able to multiply virus but when the titer in the mouse blood falls below  $10^7$  the mosquito is seldom infected (Haddow, 1955). In the mammal one obtains only a serological response after subcutaneous inoculation of  $10^{2.2}$  LD<sub>50</sub> of EEE, but fatal infections follow the injection of more than  $10^{3.3}$  LD<sub>50</sub>. The workers in the Communicable Disease Center of the U.S. Public Health Service at Montgomery, Alabama, have shown that different species of birds circulate very different amounts of virus in the blood (Chamberlain *et al.*, 1954). So it becomes possible to arrange them in a sequence of increasing epidemiological potential, a concept which, when it can be studied in this way, may well have implications for phytopathogenic viruses also.

One of the epidemiological implications of this mechanism of transmission is that the mosquitoes remain infectious for their duration of life and thus may constitute reservoirs of disease. However, this alone does not provide a mechanism for the survival of the virus from one epidemic to the next. The role of other biting insects (especially Diptera) in the epidemiology has never been adequately examined and the recent suggestions of persistent viremia in bats (Corristan, LaMotte, and Smith, 1956) indicate just how little we know of the ecology of even the most studied encephalitides.

Yellow fever and others of the viral encephalitides can pass from the larva through the pupal stage to the adult, but no example of infection in mosquitoes from adult to adult via the egg has been confirmed,—although there are reports of transovarial passage of these same viruses in the Acarina. One intriguing possibility is that the virus may conceivably infect certain of the arthropod parasites of bats, and these with their highly developed viviparity, might be well adapted to transmission of the virus hereditarily.

In distinction to the details of transmission I have just described are those exhibited by the pox virus e.g., fowl pox, bird pox, rabbit fibroma and myxomatosis, etc. With these, transmission has been shown to be purely mechanical (Day, Fenner, and Woodroffe, 1956).

Although there has been some reluctance on the part of other workers to accept our view that the mosquito in transmitting myxomatosis is involved in the manner of a “flying pin”, we have now assembled a variety of evidence demonstrating beyond reasonable doubt that transmission is purely mechanical. The following lines of evidence bear on this problem.

- a. Virus is acquired from skin lesions and not from blood. Ingested virus in the midgut does not induce infection.
- b. Virus injected into the hemocoel does not multiply.
- c. There is no latent period between the acquisition feed and the ability of the mosquito to transmit, and interrupted feeding usually causes infection.
- d. Virus concentration in the mosquito decreases with time.
- e. The probability that a mosquito will cause infection decreases with each probe.
- f. Vector specificity is not marked.

In every particular these results differ from those obtained in comparable experiments with the viral encephalitides and the conclusion is inescapable that mosquitoes are able to transmit viruses by two distinct mechanisms.

Based on the hypothesis of mechanical transmission, it has been possible to derive an estimate of the mean minimum virus load acquired by a mosquito during an acquisition feed, the number of virus particles wiped off at each subsequent feed, and the number lost per day in other ways. The method is as follows:

The shaved backs of rabbits are marked off in checker board fashion and infected mosquitoes permitted to probe on a number of successive sites. Local lesions can be detected in 3 to 5 days. Summing a number of these gives a probability of infection for each probe,

and the logarithm of this probability is found to decrease linearly. Appropriate statistical analyses by methods developed by G. A. McIntyre of the C.S.I.R.O., Division of Mathematical Statistics, have permitted the estimation of the parameters mentioned above. The results of a large series of tests has indicated that a mosquito is unlikely to acquire virus unless the skin titer is in excess of  $10^7$  LD<sub>50</sub>/gm of wet tissue. It is to be noted that many of the mechanically transmitted viruses characteristically multiply in the skin or epidermal tissues. In myxomatosis the virus is aggregated in certain cells and the amount of virus acquired by individual mosquitoes varies within very wide limits.

It will be apparent that this mechanism of transmission has many of the characteristics of the non-persistent aphid-borne viruses, except that in these decrease in infectivity is much more rapid (Day and Irzykiewicz, 1954). These viruses have been termed "non-persistent", but that term is inappropriate if applied to myxomatosis which may persist for 6 months on the stylets of the vector.

Recent work by Chamberlain has demonstrated that EEE may be transmitted by both the mechanical and the biological mechanisms by the same mosquito. During the first few days after probing a mosquito transmits EEE as do pins jabbed under the skin of an infected chick. Similarly, *Stomoxys* transmitted the virus up to 4 hours but not 24 hours after an acquisition feed. However, EEE is an unusually invasive virus which builds to high titers. Furthermore, mosquitoes show a disinclination to feed for a short time after an acquisition feed so that mechanical transmission may well play only a minor role, if any, in the epidemiology of the encephalitides in nature (Chamberlain, personal communication).

It is apparent that different viruses have different tissue affinities, that they may grow preferentially in some tissues and reach higher titers in some tissues than in others. Each of these characteristics can have an effect on transmission. It is a useful generalization that mechanically transmitted viruses are characteristically viruses of the epidermal tissues, whereas the biologically transmitted viruses are more often found in the vascular systems. Also, that mechanically transmitted viruses may be transmitted by a wider variety of vectors than biologically transmitted viruses.

I should like to conclude by suggesting that there are plenty of intriguing problems for entomologists in the field of viruses and I think they are of sufficient general biological interest and potential economic significance that those of us in the field would appreciate all the help from other disciplines that we can get. For example, we need culture methods for Simuliidae, hippoboscids and other blood sucking Diptera. We need data on the physiology of salivary secretion, gut absorption (particularly the permeability to large molecules and viruses), an examination of "interference" in vectors, insect tissue cultures, and so on.

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## DISCUSSION

R. H. BRADLEY. Is the concentration of myxoma virus in the blood of infected rabbits great enough to infect mosquitoes that ingest the blood?

M. F. DAY. Myxoma virus may be present in the white cells of an infected rabbit and in concentration to infect mosquitoes if they could acquire it from these cells.

T. E. MITTLER. Is there a decrease in infectivity of 'non-persistent' viruses in mosquitoes that feed on sugar or nectar droplets?

M. F. DAY. There is some but it is not as rapid as when the mosquitoes probe the skin of rabbits.

L. A. TERZIAN. In mosquitoes that you inoculated the haemolymph with virus, I assume that you examined the salivary glands but found no virus.

M. F. DAY. It did not seem necessary to do this, because the virus concentration in the whole mosquito declined rapidly.

E. S. SYLVESTER. Does mechanically transmissible myxoma virus inoculated to mosquitoes' blood survive long?

M. F. DAY. About as long as it does in serum in the laboratory.



# The Specificity of Transmission of Some Non-Persistent Viruses

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## ABSTRACT

When leaves infected with potato virus Y and other non-persistent viruses were exposed to ultraviolet light the infectivity of their expressed saps was reduced to about one fifth of that of the controls, suggesting that the viruses were much more concentrated in the epidermis than elsewhere in the leaves.

Non-persistent viruses are usually transmitted by aphids much more readily after about two minutes feeding on infected leaves than after longer times. With irradiated leaves there was no greater ability after short than after long infection feeding times.

*Brevicoryne brassicae* (L) does not transmit cauliflower mosaic virus optimally after 2 minutes infection feeding, and its ability to transmit is not affected by irradiation of the infected leaf on which it feeds. *Myzus persicae* (Sulz) on the other hand transmits cauliflower mosaic in the same manner as other non-persistent viruses and irradiation reduces its ability to transmit after short infection feeds.

Potato virus C is serologically related to potato virus Y and both are similarly affected by ultraviolet irradiation. But potato virus Y is readily aphid-transmissible and potato virus C, according to previous workers, not at all.

A culture of potato virus C maintained for 8 years in *Nicotiana glutinosa* became transmissible by *Myzus persicae*, though less readily than potato virus Y. When inoculated to Majestic potato and returned to tobacco this culture usually again reverted to one not transmitted by aphids. The ability of a virus to be transmitted by an aphid cannot be correlated with any known physical or chemical property; nor with its distribution in the leaf or susceptibility to secretions by aphids. Present evidence suggests that it perhaps depends on the virus particle having some special group, probably only a small part of its total constitution, that combines specifically with some component of the aphid's mouthparts.

This paper deals with viruses that resemble henbane mosaic virus in the way they are transmitted by insects. The term "non-persistent" includes them, but has also a wider connotation. They include henbane mosaic, potato Y, cucumber 1, Severe Etch, Cabbage Black ring-spot, sugar beet mosaic, and some other viruses with similar characteristics and behaviour. These are all transmissible mechanically by pricking or rubbing infected sap into healthy leaves, and their insect vectors are aphids. When vectors are starved before feeding on the infected plants they transmit most successfully after only one or two minutes feeding, and when the infection feed is prolonged their infectivity decreases to a minimum, sometimes only a tenth of their initial infectivity, after 1 or 2 hours. Unstarved aphids transmit less often and their ability to transmit does not vary with the length of infection feeding time (Watson & Roberts, 1939).

The rapidity of transmission of these viruses, the ease with which they are sap-transmissible and the lack of a latent period in the vectors point to a simple method of transmission, namely that virus particles adhere to the aphid's stylets during the infection feed, and are rubbed off into healthy tissues during the test feed, infection being caused in the same way by aphids as by pricking with a needle. However the effect of fasting, and the decline in infectivity of the vectors while feeding on infected plants suggest that transmission is affected by the physiological condition of the aphids. Also the aphids are selective in their ability to transmit; some species fail to transmit certain viruses although they can transmit others from the same host. *Myzus ornatus* (Laing), for instance, can transmit cauliflower mosaic but not Cabbage black ring-spot virus (Kvicala, 1945). The quantitative effect of fasting on the vectors' infectivity varies with different viruses and with different vectors of the same virus.

Furthermore many species of biting and sucking insects feed on the hosts of these viruses without transmitting them, and aphids feed on hosts of many other viruses which they cannot transmit. Some of the viruses that appear not to be aphid-transmissible are among the most stable and most highly concentrated in infected leaves. Tobacco mosaic

virus, for instance, is from 100 to 1000 times more concentrated in the leaf than henbane mosaic virus, and yet *Myzus persicae* if fed on a leaf containing both henbane mosaic and tobacco mosaic viruses transmits only henbane mosaic.

So far there is no satisfactory way of reconciling the apparent lack of a biological relationship between the henbane mosaic group of viruses and their vectors, with the effect of preliminary fasting of the vectors, and the relatively high degree of specificity exhibited in transmission. This is presumably because we do not know the necessary facts, or have misinterpreted them.

When aphids feed for only a few minutes on leaves their stylets penetrate no further than the epidermis (Roberts, 1940). Their high infectivity after a few minutes feeding and the later decrease could therefore be explained if most of the virus occurs in those cells. Infected leaves can be exposed to ultraviolet light in such a way that most of the virus in the epidermis is inactivated but little of that in the deeper tissues. When this is done the infectivity of sap from infected leaves is greatly reduced (Bawden, Hamlyn & Watson, 1954). Table I compares the mean number of starch lesions per half leaf (Holmes, 1931) produced by viruses coming from irradiated and unirradiated leaves.

TABLE I. Effect of Irradiation of Leaves Infected with Non-persistent Viruses, on the Numbers of Starch Lesions per Half Leaf Caused on Healthy Plants.

Numbers of Starch Lesions per Plant Leaf Surface					
Data expressed as mean log (N + 10)					
Dilution	Untreated		Irradiated		S.E.
	1/50	1/500	1/10	1/100	
Henbena mosaic virus	2.21	1.60	2.00	1.50	0.035
Cucumber virus 1	1.75	1.28	1.69	1.33	0.041
Potato virus Y	2.06	1.54	1.89	1.33	0.058
Severe Etch virus	1.94	1.44	1.92	1.46	0.176

In all, infectivity was reduced to about 1/5 of that of the controls. The volume of epidermis cannot be more than 1/5 of the whole leaf, but it apparently contains nearly 4/5 of the virus. Therefore when the aphids are feeding from epidermis they are probably tapping a much more concentrated source of virus than at other times.

When aphids are starved and then fed for short times on irradiated leaves their ability to infect becomes less than if they were fed for 24 hours on unirradiated leaves (Table II).

TABLE II. Transmission of Non-persistent Viruses from Irradiated and Unirradiated Infected Leaves. Data Expressed as Angular Transformation of Proportion of Plants Infected by Previously Starved *Myzus persicae* (Sulz), 3 Aphids per Plant.

Infection feed	Untreated		Irradiated		S.E.
	2 min.	24 hr.	2 min.	24 hr.	
Henbane mosaic virus	90	59	7	29	5.32
Cucumber virus 1	77	24	12	7	5.79
Potato virus Y	74	17	10	12	3.67
Severe Etch virus	72	20	20	7	9.35

This suggests that most of the virus acquired by vectors of the henbane mosaic group of viruses comes from epidermal cells, even that which they transmit after 24 hours on the infected plants. But there is indication of a few becoming infective by feeding on other parts of the plant, and the following results (Table III) comparing the transmission of Cabbage black ring-spot virus and cauliflower mosaic by *M. persicae* and *Brevicoryne brassicae* (L) suggest that both viruses and vectors may differ in the extent to which virus from the epidermis is transmitted. Both viruses were obtained from turnip plants, so the conditions were equivalent, but *B. brassicae* transmitted Cabbage black ring-spot optimally after 2 minutes feeding, and cauliflower mosaic after 24 hours (see also van Hoof, 1954). Moreover irradiation had little effect on the optimum infectivity of *B. brassicae* trans-

TABLE III. Transmission of Cauliflower Mosaic and Cabbage Black Ring-spot Viruses by Previously Starved *M. persicae* and *B. brassicae*. Cauliflower Mosaic Virus Data as Mean Angular Transformation of Proportion of Plants Infected by 3 Aphids per Plant. Cabbage Black Ring-spot Virus Data as Number of Local Lesions per 100 Aphids.

Infection feeding time	Unirradiated leaves		Irradiated leaves		S.E.
	2 min.	24 hr.	2 min.	24 hr.	
<i>M. persicae</i>					
Cauliflower mosaic virus	27	13	8	11	3.93
Cabbage black ring-spot virus	101	17	9	14	6.97
<i>B. brassicae</i>					
Cauliflower mosaic virus	38	64	22	52	6.01
Cabbage black ring-spot virus	30	11	6	6	3.55

mitting cauliflower mosaic, suggesting that the main source of infection was not the epidermis.

The explanation of the effect of very short feeding with most of the viruses, could be that the aphids at first pick up sap with a high concentration of virus which later pick up sap containing less. The behaviour of *B. brassicae* with cauliflower mosaic is difficult to explain on this hypothesis. The virus is obviously present in the epidermis, because *M. persicae* picks it up there, and *B. brassicae* can transmit from the epidermis because it does so with the Cabbage black ring-spot. On the evidence it seems that virus is available to aphids in deeper tissues than the epidermis though usually in too small an amount to influence transmission, but that some other factor as well as distribution affects its transmission. This factor could well be an inhibitor produced by the aphids during feeding, which might be produced by different aphids in varying quantities and at different times after starting to feed. This could account for the effect of fasting, and for some of the observed variations in the behaviour of different aphids, but it would have to be quite unprecedentedly complex and specific as an inhibitor if it is to account for everything that happens.

Besides, the inhibitor could not account for the failure of aphids to transmit tobacco mosaic virus, which is known to be highly concentrated in the epidermis, if it is also to account for the effect of fasting. The hypothesis supposes that it is not produced for the first few minutes of feeding, and tobacco mosaic virus could presumably be transmitted at that time as in henbane mosaic virus. Even if there were another inhibitor, produced continuously whether the aphid fasted or not, it would have to be a very unusual substance to inactivate tobacco mosaic virus and not affect henbane mosaic.

There are examples in the literature of insect-transmissible viruses which have lost the power to be insect-transmitted. It seemed of interest to obtain one of these and discover if any other character which might affect its insect transmissibility had altered at the same time. Potato virus C (Bawden, 1936; Cockerham, 1943), has long been recognised as a non-aphid-transmissible strain of potato virus Y, and was particularly suitable to the investigation because it has been so carefully tested by a number of workers. The first thing to test was whether its distribution in the thickness of the leaf was the same as potato virus Y. Irradiation tests using both viruses in tobacco plants, gave the local lesion counts shown in Table IV. The reduction of virus concentration by ultraviolet irradiation was the same for both viruses showing that they were similarly concentrated in the epidermis.

TABLE IV. The Effect of Ultraviolet Irradiation of Tobacco Leaves Infected with Potato Viruses Y and C on Number of Starch Lesions per 1/2 Leaf Caused by Inoculating Freshly Extracted Sap to Healthy Tobacco, Mean log (n+10).

	Unirradiated		Irradiated		S.E.
	1/25	1/250	1/5	1/50	
Potato virus Y, dilution	2.14	1.44	2.17	1.71	0.045*
Potato virus C, dilution	1/5	1/50	1/1	1/10	0.060*
	1.95	1.22	1.80	1.51	

\*Pooled errors.

The source of potato virus C used in those experiments was an infected clone of Edgecote Purple potatoes. About 1,000 *M. persicae* were used to test its insect transmissibility in tobacco plants without success, although potato Y was repeatedly transmitted by many fewer aphids.

However another source of potato virus C (Watson, 1956) was tested and most unexpectedly this turned out to be aphid-transmissible, although the same virus culture had eight years previously been convincingly shown not to be so (Bawden & Kassanis, 1947). Between those tests in 1947 and the present ones it had been maintained by sap inoculation through a series of subcultures in *Nicotiana glutinosa* plants. These were used as a source of infection and about 1 in 20 aphids transmitted it whereas the potato Y in the same conditions 1 in 2 aphids transmitted. When the virus from *N. glutinosa*, after being transmitted by aphids to tobacco plants, was re-inoculated into Majestic or President potatoes it caused only the local necrotic lesions characteristic of potato virus C and no systemic infection. Both isolates could be taken back from the infected potato leaflets to tobacco plants, and when this was done the isolate from *N. glutinosa* was no longer aphid-transmissible; it then resembled the Edgecote Purple virus C in every respect. In several repetitions of the experiment, the virus occasionally remained aphid-transmissible after one passage through potato, and very rarely after two passages; eventually all the viruses which were passed through potato became non-aphid transmissible.

It seems almost certain that the original isolate of virus C had undergone some change that turned it into an aphid-transmissible virus during its sojourn in *N. glutinosa*. The fact that the change was reversed by passage through potato suggests that it was qualitative, and that the virus mutated to the transmissible form which infected *N. glutinosa* and the reverse mutation was induced by transfer through potato. Potato does not reduce the aphid-transmissibility of potato virus Y, so this behaviour is peculiar to the anomalous C virus.

The change could be interpreted as a quantitative one by assuming that, when virus C multiplies, some of the particles produced are always aphid-transmissible and some not, but that potato is so unfavourable an environment for the aphid-transmissible particles that only few are produced in it, whereas *N. glutinosa* is a favourable host, where they multiply up to a level easy to detect experimentally.

Whatever the actual mechanism of the change the ability of a virus to be transmitted by aphids is demonstrably a property of the virus particle, genetically determined and linked with other inherent properties. The behaviour cannot be explained as a simple effect of distribution in the leaf because both transmissible and non-transmissible strains of virus Y appear to have the same distribution. One strain and not the other might conceivably be affected by an inhibitor produced by aphids while feeding, as has been postulated, but it is unlikely that there are inhibitors of sufficient complexity and specificity to account for all the specificity which exists among aphid-transmitted viruses and between these and other viruses.

When bacteriophage invades a bacterium it first adsorbs onto the surface of the host cell. Phages which cannot invade the bacterium cannot adsorb either, but influenza virus will adsorb onto the surface of red blood corpuscles without invading. Some insect-transmitted viruses invade the tissues of their insect hosts. Others, possibly, are carried on the surfaces of cells in the blood without invading them. There is no intrinsic objection to the possibility that still others adsorb temporarily, but specifically, to surfaces in the pharyngeal area of the aphid's foregut. However Bradley & Ganong, 1955, showed that destroying the active potato Y virus at the tips of the stylets of viruliferous aphids rendered the aphids almost completely non-infective. This seems to be conclusive evidence that the virus is carried in that region and to preclude the possibility of a living surface being involved, for the stylets, so far as is known, are composed of chitin and bare of living tissue.

According to Frazer, 1944, the hemipteran-transmitted fungus, *Nematospora gossypii*, which causes internal boll disease of cotton, is carried by the vector, *Dysdercus intermedius*, in the sheaths which surround the bases of the stylets when they are retracted into the head. The fungus spores reach the stylet sheaths, and are returned to the stylet channel, by leaking between the maxillae at the point where these mouth parts come together to



enclose the anterior end of the pharynx. The maxillae are apposed in this region by muscles, which relax when the mouthparts are inserted into or withdrawn from, the leaf (Tower, 1914). There is evidence that non-persistent viruses are transmitted by aphids mainly at the time of penetration or withdrawal of the stylets, and it is conceivable that some leakage of the kind demonstrated for transmission of cotton boll disease might be involved, though it is difficult to reconcile this with the conception that only the tips of the stylets are involved in transmission. However it is almost equally difficult to reconcile the kind of specificity exhibited in the transmission of potato virus Y and potato virus C, with the simplicity of the mechanism by which they appear to be transmitted.

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# Studies of Aster Yellows Virus Transmission by the Leafhopper Species *Macrosteles fascifrons* Stål and *M. laevis* Ribaut

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## ABSTRACT

The specificity of aster yellows virus transmission was tested with leafhopper species *M. fascifrons* from U.S.A. and *M. laevis* from Europe. Colonies of virus-free insects were reared on rye and wheat plants. In 5 consecutive tests a total of 200 American and 200 European leafhoppers from stock were inoculated by feeding for 2 weeks on plants with either the Eastern or the California strain of yellows. Afterwards the insects were tested for 2 or more weeks, in groups of 3 to 5 per plant, on young China aster plants. None of 80 plants exposed to *M. laevis* became infected, while *M. fascifrons* transmitted the Eastern strain of virus to 38 of 40 plants, and the California strain to 23 of 40 plants.

In order to establish whether the leafhoppers constitute physiological races, varieties or different species, virgin females of *M. laevis* and *M. fascifrons* were paired with males of the opposite kind. No progeny resulted from attempted crossings and it was concluded that these leafhoppers, placed by taxonomic authorities in the *M. fascifrons* complex on the basis of male genitalia, belong to separate sibling species. Standard taxonomic criteria for the identification of Cicadellidae seem inadequate and breeding tests, similar to those developed for Lepidoptera, may become a useful additional means for species identification.

The tests indicate that the degree of specificity in the transmission of aster yellows virus is relatively high. The earlier belief that introduction to Europe of plants with American aster yellows would result in the spread of the disease by *M. laevis* seems unwarranted. The nature of the high specificity of vectors is not understood, but there are indications that it is caused by barriers to viral multiplication in the insects and by barriers to the penetration of the gut wall and the salivary glands. It is hoped that improved tissue culture and cytological techniques will clarify the nature of vector specificity.

The Eastern and California strains of aster yellows were shown by Kunkel to interfere in plants and in *M. fascifrons* but no similar data are available for other yellows-type viruses. In the absence of such experiments and of serological tests for this group it cannot be established whether different strains or different distinct viruses are responsible for yellows syndromes in other areas of the world. The ability of several species of leafhoppers to transmit yellows does not contradict or breakdown the concept of specificity; the search for vectors of typical yellows-type viruses can probably be limited to the Cicadellidae at present.

## INTRODUCTION

In the United States only one *Macrosteles* species, *M. fascifrons* Stål, is known as a vector of the aster yellows virus, *Chlorogenus callistephi* var. *vulgaris* Holmes. In California several additional species of leafhoppers are reported to transmit the California strain of aster yellows, *Chlorogenus callistephi* var. *californicus* Holmes (Day and Bennett, 1954). Other species of leafhoppers have been listed as vectors of aster yellows virus in other areas (Köhler and Klinkowski, 1954) but identity of the viruses transmitted is uncertain. In spite of several reported vectors, the degree of specificity of aster yellows virus transmission appears to be very high and it is certain that all established vectors belong to the cicadellid leafhoppers. This paper describes one of the most extreme cases of vector specificity found in testing aster leafhoppers from Europe and America for their respective abilities to transmit aster yellows virus.

## LITERATURE

In 1924 Kunkel found that aster yellows virus was transmitted by the common aster leafhopper. Specimens of the vector were sent in that year for identification to Dr. E. S. China of the British Museum and identified as *Cicadula sexnotata* Fallen. In his classical paper on aster yellows, Kunkel (1926) pointed out that the vector insect was probably similar to the *C. sexnotata* of Europe. The late Dr. Herbert Osborn suggested that the

common aster leafhopper may have been introduced into the New World in the second half of the XIXth century, because this species was not mentioned from the United States prior to that time despite rather extensive collections of leafhoppers in America. At present the aster leafhopper is very common throughout the United States and Canada. The aster yellows disease is also widely distributed. The California strain causes severe damage to economic plants in Saskatchewan (Sackston, personal communication) and its occurrence in the eastern United States (Pennsylvania, for instance) (Kunkel, personal communication) established in recent years, constitutes a serious threat to celery growers.

Diseases called aster yellows have been described also from other parts of the world. In 1930 Fukushi described aster yellows in Japan. In 1936 Richter reported its occurrence in Germany. Suchow and Vovk found it is spread by a *Macrostelus* species in Russia (1945). Blattny *et al.* (1956) reported it from Czechoslovakia, Frazier and Posnette from England (1956); its presence in Hungary, Bermuda, Indonesia, and Mexico, is suspected. Establishment of the identity or possible close relationships between the causative viruses is of great practical and scientific importance. It could provide an answer to the question of the origin of aster yellows virus and explain why it is rare in Europe and common in America. Is the same species of *Macrostelus* responsible for virus transmission on the American and European continents? Could the American strains of the virus become established in Europe if accidentally introduced? Or do they already occur there? If the American virus differs from the European, would the reciprocal introduction of viruses be dangerous, or would there be no potential specific vectors present to transmit the causative agents? Is the American leafhopper a native species or one introduced recently?

The present paper presents an attempt to clarify some of these problems by the study of aster leafhoppers from America and Europe and by a comparison of their abilities to transmit aster yellows virus.

## MATERIALS AND METHODS

In October, 1954, living stems of an unidentified grass were sent from Europe for cytological studies. These stems were almost completely dry on arrival and the tissues were dead a few days later. As the recipient was about to discard what was left of this material after his cytological studies, he noticed that 2 leafhopper nymphs had hatched from the stems which had been kept in a moist chamber. These first-instar nymphs were transferred to young rye seedlings (*Secale cereale* L.) and caged in an isolation greenhouse insectary in New York City. They molted a few times and eventually became adults. One turned out to be a male and the other a female. The insects closely resembled the common American aster leafhopper *M. fascifrons*. Frequent transfers and special care were given the progeny of this pair of leafhoppers during the winter months 1954–1955. Insect cages, described previously (Maramorosch, 1951a, 1951b, 1953) were used for rearing and for transmission tests. In the spring of 1955 a fairly large colony was at hand to start transmission tests and to attempt identification.

## IDENTIFICATION OF THE LEAFHOPPERS

Specimens of the newly obtained insects as well as of American aster leafhoppers maintained since 1924 in a stock colony by Dr. L. O. Kunkel were sent for identification to Dr. E. S. China at the British Museum in London, England, to Dr. E. Wagner in Hamburg-Fuhlbuttel, Germany, to The Science Service, Ottawa, Canada, and to the Insect Identification and Parasite Introduction Section, U.S. National Museum, Washington, D.C. The author wishes to acknowledge with thanks the helpful cooperation of those who identified the insects. The taxonomists in four countries faced a difficult task, particularly in view of the fact that the origin of the insects was not divulged and the specimens of leafhoppers—one or two of each kind—were only distinguished by code letters.

At the U.S. National Museum males of the American colony were identified in 1952 as *M. fascifrons*. In 1955 2 males of the European colony were also found to be *M. fascifrons*.

The Science Service in Ottawa identified in 1952 insects from the American colony as *M. fascifrons*. In 1955 2 males of the new colony were recognized as the same species. An additional note read: "This variety, in which the posterior pair of spots of the vertex are greatly enlarged whereas the median pair are virtually absent, is rare though specimens have previously been seen".



Dr. China received in 1955 one European and one American insect. A possible difference between the 2 individuals was mentioned in the accompanying letter and it was stated that one of the insects descended from the original colony identified by Dr. China in 1924. In his reply Dr. China noted the similarity of the insects, but pointed to the following differences: Insect B (European colony) had narrower blades to the apex of the aedeagus, it had "The spotting of the head and scutellum typical of *M. laevis* Ribaut. It runs down in Dorst's key to *M. lepidus* (VanDuz.) but the genitalia are, of course, quite different from those of that species". The leafhopper C (American), on the other hand, "has the head spots typical of *M. divisus* (Uhler) but the aedeagus is identical with that of *M. laevis* Ribaut and possesses the broader blades typical of that species". . . . "You do not say whether it is B or C which is descended from the original colony but I am inclined to regard C as the specimen while B is of European origin. Am I right?" The conclusion was correct because B was used as code letter for the European specimen.

Dr. Wagner based his identification mainly on the form of the penis and called specimen B *M. laevis* Rib.; he stated also that this species is common in Europe, but, as far as he knew, was not previously found in America. He therefore requested the exact location and plants from which the insects were collected. Specimen C was classified as fitting the earlier description of *M. wilburi* Dorst, which has been placed in the *M. fascifrons* complex by Beirne (1952). According to Dr. Wagner, representatives of this group have not, so far, been found in Europe.

The results of the four identifications are summarized in Table I.

TABLE I—Identification of Aster Leafhoppers.

Place of identification	Origin of colony	
	U.S.A.	EUROPE
Washington, U.S.A.	<i>M. fascifrons</i> Stål (1953)	<i>M. fascifrons</i> Stål (1955)
Ottawa, Canada	<i>M. fascifrons</i> Stål (1953)	<i>M. fascifrons</i> Stål (1955)
London, England	<i>C. sexnotata</i> Fall. (1924)	
	<i>M. laevis</i> Ribaut = <i>M. divisus</i> Uhler (American) (1955)	<i>M. laevis</i> Ribaut = <i>M. divisus</i> Uhler (European) (1955)
Hamburg, Germany	<i>M. fascifrons</i> Stål = ( <i>M. wilburi</i> Dorst) (American (1955)	<i>M. laevis</i> Ribaut identical with the European species (1955)

BREEDING TESTS

From the identification in Table I it can be seen that the presently used criteria for leafhopper identification make it difficult to separate or distinguish the aster leafhoppers of Europe and America. While one taxonomist designated them as separate species, other authorities considered them as either varieties of the same species or as representatives of a single insect complex. Breeding tests were therefore undertaken to clear up the question of the identity of European and American insects. It was hoped that the results of such tests would establish whether the leafhoppers represent two different species or perhaps constitute varieties or physiologic races of *M. fascifrons*.

The sexual compatibility of males and females of the two colonies was tested in the following way. Fifty last-instar nymphs of each group were caged singly in leaf cages (Maramorosch, 1951a) on aster plants for one week. Almost all nymphs molted and virgin females thus obtained were used for the tests. In addition, a faster method was used for obtaining other virgin females. It employed a technique developed in Dr. L. M. Black's laboratory by Mrs. Shirley Teitelbaum in 1949 (personal communication) for the sexing of nymphs of *Agallia constricta*. This faster method employed a visible difference in the shape of the segments of the abdomen in last-instar nymphs. Sexing was carried out under CO<sub>2</sub> anesthesia and a binocular microscope (Maramorosch, 1956a). Female nymphs were placed on a single plant and a few days later the adult virgin females were used for breeding tests.

In the first test 5 females of the American colony and 5 males from the European colony were caged in pairs on 5 large aster plants. Another set consisted of 5 pairs of females from the European colony paired with males of the American stock. As controls, 2 sets of 5 homologous pairs were caged on 10 aster plants. The results are presented in Table II. Although average numbers of progeny were obtained from homologous control pairs, no progeny resulted from crosses between the American and European leafhoppers.

The tests were repeated with certain modifications. Instead of individual pairs, 2 groups of 20 males and 20 females from each colony were placed on rye plants in 2 separate breeding cages. The insects were transferred once a week for 6 weeks to fresh rye plants. No progeny resulted from these attempted crossings and it was concluded that the European and American leafhoppers belong to separate species.

TABLE II—Cross-breeding Tests of American (*M. fascifrons* Stål) and European (*M. laevis* Ribaut) Leafhoppers.

Pair No.	M.f.   x   M.l.	Progeny	M.f. x M.f.	Progeny
1.	♂       ♀	0	1.	23
2.	♂       ♀	0	2.	42
3.	♂       ♀	0	3.	30
4.	♂       ♀	0	4.	15
5.	♂       ♀	0	5.	41
6.	♀       ♂	0	M.l. x M.l. 1.	15
7.	♀       ♂	0	2.	9
8.	♀       ♂	0	3.	14
9.	♀       ♂	0	4.	21
10.	♀       ♂	0	5.	20

In addition to individual pairs, two groups of 20 males and 20 females of *M. fascifrons* and *M. laevis*, respectively, were set up on wheat plants for 6 weeks. No progeny resulted.

TRANSMISSION TESTS

Experiments were designed to test the specificity of aster yellows virus transmission by the 2 species *M. fascifrons* and *M. laevis*. These species seemed ideally suited for a comparison in their ability to transmit the virus, because both were known as vectors of viruses causing aster yellows in their native countries. Furthermore, the insects were almost indistinguishable on the basis of male genitalia and were placed by all but one taxonomic authority in the *M. fascifrons* complex. Colonies of virus-free insects of both species were reared on rye (*Secale cereale* L.) and wheat (*Triticum sativum* L.) plants. Separate colonies of each were exposed to both strains of aster yellows virus by feeding for 2 weeks on aster plants with either the Eastern or the California disease. Insects thus exposed were transferred to young China aster (*Callistephus chinensis* Nees) plants for a period of 4 weeks, in groups of 3 to 5 insects per plant. In 5 consecutive tests a total of 200 European and 200 American insects were tested. The results are summarized in Table III. The Eastern strain of virus was transmitted to 38 of 40 exposed aster plants, and the California strain to 23 of 40 plants by the American insects. Neither strain of virus was transmitted by the European insects. It was concluded that the European aster leafhopper, in spite of its very close resemblance to the American leafhopper, is unable to act as vector of the American strains of aster yellows virus.

TABLE III—Transmission Tests of Aster Yellows Virus with American and European Aster Leafhoppers\*.

Virus	American insects Plants infected/exposed	European insects Plants infected/exposed
Eastern strain of aster yellows virus	38/40	0/40
California strain of aster yellows virus	23/40	0/40

\*Summarized results of four tests in which 200 American and 200 European leafhoppers were inoculated by feeding on diseased aster plants for 2 weeks, then tested for 4 weeks in groups of 3 to 5 insects per plant, on 160 China asters. Noninoculated controls from the same colonies proved virus-free.

### DISCUSSION OF RESULTS

*M. fascifrons* and *M. laevis* are vectors of very similar viruses. The insects seem so closely related by intergradation in the morphologic characters as to suggest that they evolved from the same species complex. The American aster leafhopper probably originated in Europe and was introduced to the western hemisphere in the XIXth century, as suggested by Osborn (Kunkel, 1926). Due to evolutionary processes in the new environment the insect may have developed sexual incompatibility with the original European ancestor.

The European insects were unable to transmit the two known American strains of aster yellows virus. It is therefore assumed that an accidental introduction of the American virus in flower bulbs or other living plant material from the New World would not be dangerous for European agriculture because the common aster leafhopper, *M. laevis*, would not spread the virus.

The aster yellows virus reported by Heinze and Kunze (1955) to be transmitted by *M. laevis* in Germany is not identical with either one of the 2 American strains. The difference in vectors indicates that most likely another strain or another related or even unrelated yellows-type virus causes the aster yellows disease in Europe. No information is available on the transmission of European aster yellows virus by American insects. The aster yellows-like disease described from England also belongs to this group but probably is not American aster yellows. Recent studies on a yellows disease from Mexico (Maramosch, 1956b) also indicate that its causative agent is distinct from aster yellows viruses. It would be interesting to test the relationships of these aster yellows-like viruses. In 1955 Kunkel demonstrated the interference of the Eastern and California strains of aster yellows virus in plants and in *M. fascifrons*. Similar tests could show whether the viruses which cause various yellows diseases in different continents are caused by related strains or unrelated viruses.

The United States Department of Agriculture maintains a laboratory for the study of animal diseases from foreign countries, on an isolated island (Plum Island) off the east coast. It would seem desirable to create a similar isolated laboratory for the study of plant viruses and vectors from various parts of the world. Numerous problems of both basic and practical interest could be studied in such a laboratory. The interrelationships between Agallian leafhoppers and various strains of the sugarbeet curly-top virus, between many different big-bud viruses and their vectors, between certain dangerous tobacco and corn viruses can not properly be investigated at present because of the dangers involved and the necessary quarantine regulations. If such a laboratory were available, the interrelationships of aster yellows viruses could safely be studied there.

The methods for identification of Cicadellidae are not adequate at present. Additional criteria, such as physiological differences and even the ability to transmit viruses may become useful. In addition to the morphological criteria used in the past, breeding tests will be necessary for the separation of certain species. Such tests are successfully used for the study of Lepidoptera and also widely applied by *Drosophila* workers (Sokoloff, 1955).

In a personal communication Dr. Freitag pointed out that the long-winged and short-winged forms of the common aster leafhopper in California do not interbreed. The two

"forms" seem thus to present another example of sibling species. In this particular case both species transmit aster yellows virus.

The specificity of transmission of viruses by leafhoppers may vary in degree. Black (1944) described the specific transmission of two strains of potato yellow dwarf virus by two different species of Agallian leafhoppers. Storey (1932) found an active and an inactive race of *C. mbila* and was able to change inactive insects into active transmitters by puncturing their gut walls. Black (1943) studied an active and inactive race of Agallian leafhoppers. Storey (1932), Fukushi (1934), Bennett and Wallace (1938), and Black (1943) found that leafhopper species vary genetically in their ability to transmit viruses.

The transmission of aster yellows virus by *M. fascifrons* but not by the sibling species *M. laevis* is probably the most extreme case of vector specificity reported. The fact that the two insects could not easily be distinguished on the basis of usual taxonomic criteria but differed in the ability to transmit a disease agent is reminiscent of the early work on *Anopheles maculipennis* in Italy (Hackett, 1937). Mosquitoes which looked alike differed in their ability to transmit the malarian parasites; only later the difference in mosquito eggs permitted the separation of various species from the *Anopheles* complex. Perhaps extreme cases of vector specificity are not as uncommon as has been believed.

Although many species of *Macrostoteles* and of other genera have been reported as vectors of aster yellows-like viruses, no insects other than cicadellids have been proven vectors. The search for vectors of typical yellows-type viruses probably can be limited safely to the family Cicadellidae. The nature of vector specificity is not completely understood. The assumption is often made that non-vector species, closely related to vector species, have barriers in the gut and perhaps in the salivary gland that prevent the movement of virus. The multiplication of virus may be limited or may not take place in such non-vector insects. It was found that the Eastern strain of aster yellows virus causes changes in fat-body cells of aster leafhoppers, after the insects acquired virus by feeding (Littau and Maramorosch, 1956) or by injection (Maramorosch, 1956c). These changes indicate that the plant virus causes a mild insect disease. Studies are under way to find whether similar pathological changes take place in *M. laevis* inoculated with strains of virus which *M. laevis* can not transmit. It is hoped that these studies as well as the use of insect tissue culture (Maramorosch, 1956d) will further our knowledge of the intimate relationships between leafhoppers and their biologically transmitted plant viruses.

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### DISCUSSION

P. W. OMAN. The opinion was expressed that there exists many cases where sibling species are not differentiated on morphological characters but easily so by cultural methods. However, representatives of specialized populations may be less differentiated, but the criteria used for their identification breaks down when tested in connection with populations occurring over wide areas.



# The Effect of Movements of Winged Aphids on Transmission of a Nonpersistent Aphid-Borne Virus<sup>1</sup>

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## ABSTRACT

*Since winged aphids are the principal vectors of the nonpersistent potato virus Y, it seems probable that conditions which affect the flights of the aphids could be reflected in correlative shifts in transmission patterns. In order to test this hypothesis experiments were carried out under field-plot conditions in south Florida.*

*Sunflower planted as a barrier between the virus source and the crop plant reduced primary spread of virus to one-fourteenth of that where no barriers were used. The use of yellow fences and/or collards in front of sunflowers did not increase the efficiency of the barriers over one row of sunflowers used alone.*

*Infection gradients present in most fields suggest that the distance virus Y has been carried can be estimated. The gradients of infection were so well defined that use of the virus as a tracer in following aphid movements seems possible.*

## INTRODUCTION

The nonpersistent aphid-borne viruses are dependent almost exclusively upon winged aphids for dissemination. Thus, it seems possible that procedures which affect flight movements of the aphids could affect the distribution of spread. It has been shown experimentally that either spraying the weed host (nightshade) with an insecticide (parathion) or placing a row of sunflowers between the infected weeds and pepper plants can cause a reduction in spread of potato virus Y in south Florida (Simons, 1957). Both of these lines of attack are effective against primary virus spread (outside the field to inside).

Spraying infected weeds resulted in reduced primary spread probably because the aphids were not able to fly as far after having contacted the aphicide. Since susceptible plants are close together in the field this effect was not demonstrable when parathion was sprayed on the crop plants.

The principal effect of the sunflowers in reducing spread probably was in preventing infective aphids from entering the crop area. Since infectivity in aphids is lost in less than an hour and rate of loss of virus seems exponential (Simons, 1956), a temporary deterring of their flights could prove useful in limiting virus spread. Although the sunflowers reduced transmission to a level of about one-third of that found in unprotected plots, the degree of control obtained was not sufficient to be commercially useful. Other types of plant barriers were tried, therefore, in an effort to find a more efficient means of excluding the aphids from the crop area.

## EFFECT OF SEVERAL TYPES OF BARRIERS ON PRIMARY SPREAD OF POTATO VIRUS Y

The pattern of spread, which has been obtained where sunflower barriers have been tested, has indicated that the aphids which penetrated to the crop had done so by flying over the barrier rather than through it (Simons, 1957). Thus, in order to improve the efficiency of the barrier, it was decided to attempt to attract the aphids to the lower portion of the barrier. Two schemes of attack were employed: 1) Yellow colored fences were placed at the base of the sunflowers, and 2) two rows of collards (*Brassica oleracea* L. var. *acephala*) were grown in front of the sunflowers to act as a "trap crop". In all, seven different barriers were tested. They included: 1) one row of sunflowers; 2) two rows of sunflowers; 3) two rows of collards; 4) two rows of collards in front of one row of sunflowers; 5) same as treatment 4 except that a yellow plywood fence 24 inches high was placed between the sunflowers and collards; 6) same as No. 5 but with a green fence; and 7) no barrier (Fig. 1).

<sup>1</sup>Florida Agricultural Experiment Station Journal Series, No. 581.

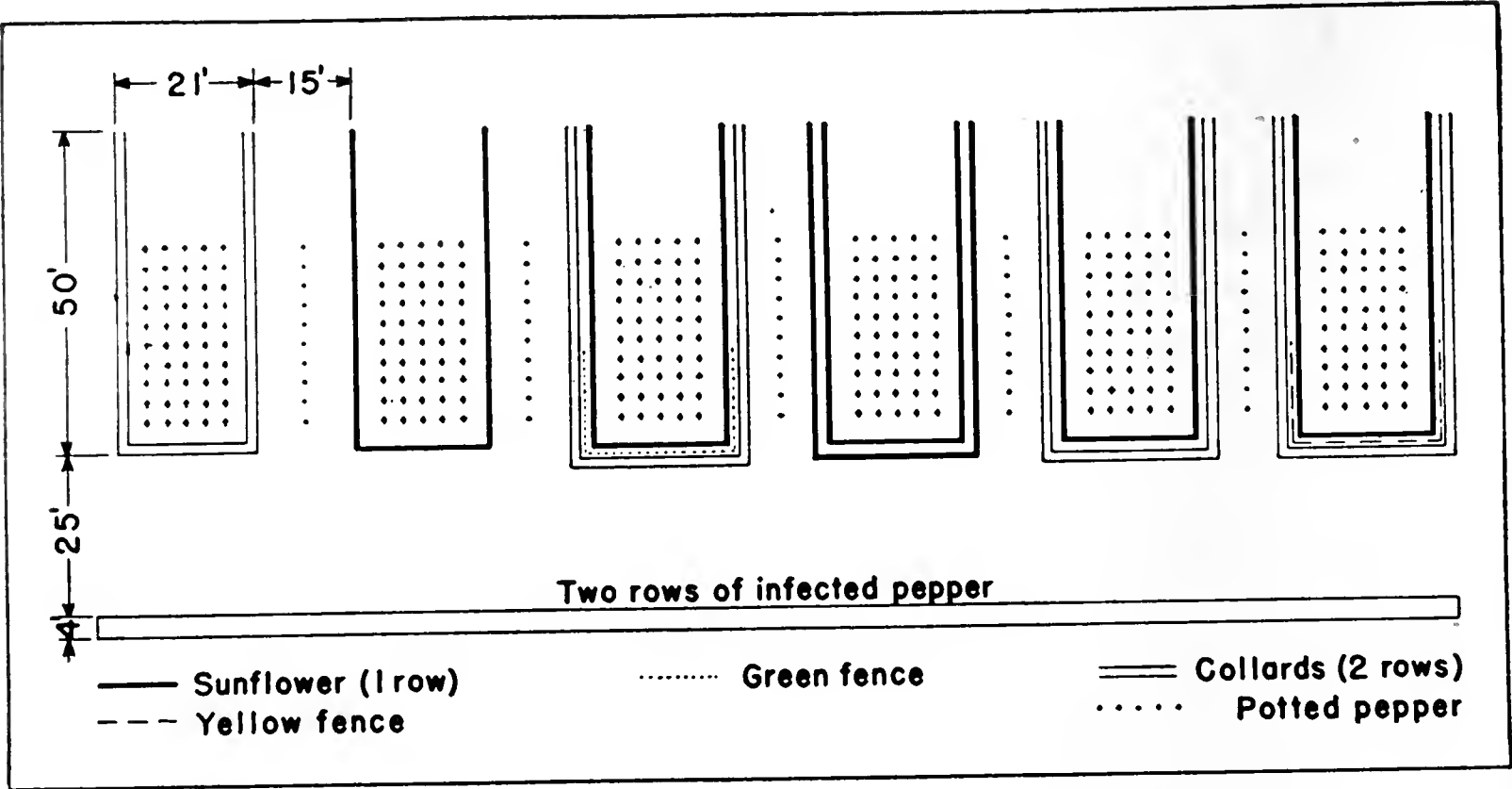


Fig. 1. Diagram showing locations of plant barriers, virus source and test plants.

Two rows of naturally infected peppers were located 25 feet in front of the barriers to provide a virus source for the aphids. In order that only primary spread would be involved, the healthy pepper (test) plants were transported in pots to the test area and left for a period of six days. At the end of this time they were removed from the field and returned to the greenhouse to complete the incubation period of the virus. Five rows of 10 plants each were transplanted in each plot excepted in the case of the check, which was comprised of one row of 10 plants placed in each of the five alleyways formed by the barriers (Fig. 1). The experiment was repeated three times so that a total of 150 plants in each treatment was used. The results are shown in Table I.

TABLE I. Effect of Several Kinds of Plant Barriers on Primary Spread of Potato Virus Y.

Treatment	Percentage disease in trial			$\bar{x}$ Trans. in per cent
	1	2	3	
No barrier	73.5	32.4	26.1	44.0
1 row sunflower	6.0	2.3	2.0	3.4**
2 row sunflower	18.0*	4.6	0.0	2.3
Sunflower + collards	2.0	4.9	0.0	2.3
Sunf. + Coll. + green fence	4.0	7.3	2.1	4.4
Sunf. + Coll. + yellow fence	4.0	2.1	4.0	3.3
2 rows collards	28.0	8.6	10.0	15.5

\*A diseased potted plant was inadvertently set out in this treatment. The results are based on trials 2 and 3.  
\*\*L.S.D. at .01 = 25.3 per cent.

All treatments resulted in a highly significant reduction in primary spread of virus over that found in the unprotected check (L.S.D. .01 = 25.3 per cent). In the case of the treatments containing sunflowers the reduction was about 14 to 1. The collards barrier gave a 3 to 1 reduction in spread which was somewhat surprising considering the fact that these plants were only one-fifth the height of the sunflowers. Since there was no indication that the collards attracted the aphids any more than did the sunflowers, it



would seem likely that more aphids flew near to the ground (from 0 to 2 ft.) than in the 2-10 ft. zone.

Neither the yellow fences nor the collards attracted sufficient numbers of flying aphids to affect transmission of virus. The sunflowers alone reduced infection to a point where any additive effect would be difficult to demonstrate. Certainly the possibility that some plant species are attractive to flying aphids does exist and the negative results obtained in this work can only be interpreted in view of the lack of effect of collards as a trap crop.

It has become increasingly apparent during field studies that virus spreads in well-defined gradients of infection. Although the gradient depends upon variables such as numbers of winged aphids present, air temperature, and proximity to and density of the primary source of inoculum (Simons, 1957), it nearly always takes a form which can be expressed as a straight line on logarithmic graph paper. In fact, the correlation between proximity to the virus source and amount of spread seems so close that it appears possible to use a virus as a "tracer" in obtaining information on certain phases of aphid flight.

Although aphid trapping is very useful in the determination of flight phenomena which are primarily physical in character, e.g., density of flight, time of day flights occur, and species of aphids present; there are definite limitations as to the type of information that can be obtained. Factors such as the distance flown per flight, relative attractiveness of a plant species to the aphid, and effect of spraying plants with pesticides on subsequent movement of aphids from these plants, cannot be readily measured by trapping. The principal problem is the inability to determine where the aphids have come from and where they are going. What is needed is a technique for "labelling" an aphid so that some of its subsequent movements can be followed. The nonpersistent aphid-borne viruses seem well-suited for this type of work.

First, nearly all aphids flying into an area are virus-free (unlabelled). Second, by using a virus which is efficiently transmitted (e.g., potato virus Y in nightshade or pepper) many of the aphids could be tagged in feeding periods of less than a minute. Third, most of the aphids lose infectivity in less than 30 minutes and would thus not cause trouble from multiple infections in the test area. The technique of using potted test plants which can be removed from the field minimizes the problem of secondary infection.

The most effective arrangement would probably involve both trapping and labelling. Through the use of such procedures it should be possible to obtain information on many facets of aphid movement, which to date have proven difficult to investigate.

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# General Review of Dust Diluents and Some of the Technical Aspects Which Affect Insecticide Application

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## ABSTRACT

*Properties of insecticide dust diluents and carriers and their use in the field of insect control are discussed from both a theoretical and practical standpoint. Diluents and carriers are discussed relative to the mineralogical group to which they belong. Important properties of the diluents and carriers are considered in light of structure of the mineral group to which they belong. Properties are also discussed as they relate to insecticide application. Specific data illustrating this is included.*

As an introduction to this discussion of insecticide dust diluents and carriers, I would like to outline briefly the points and ideas which will be considered in this paper. First, materials which are used as solid diluents and carriers will be summarized according to a scheme of classification published by Watkins and Norton (1947). After pointing out the various types of materials in use, the work of chemists, mineralogists, soil scientists, and ceramists elucidating the structure of some of these groups will be borrowed to describe the fundamental structure of these materials. Using this structural picture, then, fundamental differences between types of materials and the effect of these differences on the properties of the materials will be considered. At the same time, the importance of these properties to the use of these materials as diluents and carriers will be discussed. After this general consideration of diluents and carriers, specific data on adsorption and interaction of some chlorinated organic insecticides and related compounds will be presented.

TABLE I—Classification of Diluents and Carriers.

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Botanical flours
Minerals
Elements
Oxides
Indeterminate
Synthetics

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Table I presents a condensed form of the classification of diluents and carriers by Watkins and Norton. There is one revision from the original classification and that is the addition of a major group for synthetic materials. Under the botanical heading are such materials as soybean flours, tobacco flour, wood flours, and walnut shell flours. Synthetics comprise artificially produced materials such as precipitated calcium silicates. Table II shows a further breakdown of the oxide group of minerals. In addition to the subgroups listed the following are also found: silicon containing tripolites and diatomites; calcium, calcium and magnesium limes; carbonates, calcites and dolomites; and sulfate, gypsum.

TABLE II—Classification of Oxides of Mineral Group.

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Silicon	Silicates
Calcium	Mica
Carbonates	Vermiculite
Sulfates	Talc
Phosphates	Pyrophyllite
	Clays

Clays—Montmorillonoid, Kaolinite, Attapulgite, and Unidentified

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The types of materials used as diluents and carriers represent a very complex grouping. Not only is there a great diversity of types of materials used, but these materials may or may not be of high purity. Watkins and Norton (1947) state: "It is suggested here that for insecticidal purposes a mixture be classed with the mineral occurring in the highest percentage. This criterion will usually give a clear distinction, as in most instances one mineral definitely predominates and is responsible for most of the properties of the mixture." Since diluents and carriers will be developed on the basis of structure, it must be kept in mind that in some cases impurities or a mixture of minerals may be responsible for properties which are not in accord with the properties expected of a particular mineral group. Since a great deal of work has been done by chemists, mineralogists, soil scientists, and ceramists working out the structure of the silicate minerals and since a large majority of commercially available diluents and carriers belong to this group of minerals the structure of the following members of the silicates will be developed: pyrophyllite, talc, montmorillonoid, kaolinite, and attapulgite. In addition a member of the silicon oxide group, diatomite, will be included.

Fig. 1 is a diagram of the structure of pyrophyllite. It can be used to discuss the structure of three of the mineral groups that will be developed. These are pyrophyllite, talc, and montmorillonoid. The figure shows that the structure is made up of a combination of silicon, aluminum, oxygen, and hydroxyl groups. Silicon and oxygen atoms combine in a tetrahedral coordination to form what is called the silica sheet. Aluminum, oxygen, and hydroxyl groups combine in octahedral coordination to form the gibbsite or aluminum sheet. These two sheets form the fundamental layer as pictured in the figure. This layer is continuous in the *b* axis and in a plane perpendicular to that of the diagram. Layers stack upon one another in the *c* axis. A partial second layer is included in the diagram. It would take approximately 5,400 of these individual layers stacked one upon another to make a particle which would be 10 microns in thickness. The structure as diagrammed is that of pyrophyllite. Talc has the same basic structure except that in place of the aluminum ions in the middle of the layer there are magnesium ions. Since ions located in the center of the layer can have little effect on the properties, talc and pyrophyllite are very similar. Experience in the use of these two types of materials has shown that pyrophyllite is much more abrasive than talc. The difference can be attributed to impurities in pyrophyllite, as pointed out by Gooden (1947), such as quartz and micas. Further points on the structure of talc and pyrophyllite should be considered. The lattice structure is essentially neutral with no charge at the surface of the layers. Individual layers, stacked one upon another, present a surface of oxygen atoms of one layer to a surface of oxygen atoms of the layer above or below it. There is no binding force, then, from one layer to another and these two minerals are platy, flaky, and soft. Unsatisfied valencies or broken bonds occur when the lattice is fractured and give rise to a small exchange capacity.

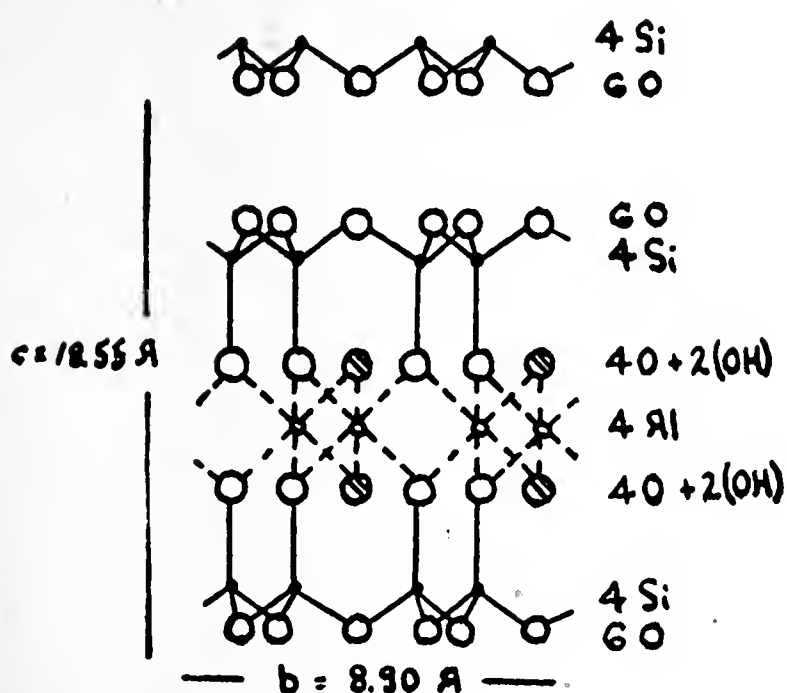
The montmorillonoid structure can be discussed using the structure of pyrophyllite. The structure is similar, except that in the montmorillonoids there is a large amount of isomorphic substitution in the lattice—that is, other ions replace Si and Al in the structure. This isomorphic substitution gives rise to a net negative charge which is manifested at the surface of the individual layers. The charge at the surface is satisfied by cations located between the individual layers. The result is an expanding *c*-axis which means that polar compounds can penetrate between the layers and expand the lattice. Ions between the layers are exchangeable and give montmorillonoids a large cation exchange capacity. Furthermore, this structure exhibits two types of surface area: one, the external area of particles; the other, the internal surface area between layers available to polar compounds.

Fig. 2 diagrams the basic structure of kaolinite. This differs from the preceding figure in that there is only one silica sheet and one aluminum sheet to a layer. Again particles consist of layers stacked one upon another. Here, however, a surface of oxygens of one layer contacts a surface of hydroxyls of another layer. There is, then, hydrogen bonding between the layers. There is no isomorphic substitution in the lattice and the lattice is neutral. Exchange capacity arises from broken bonds or unsatisfied valencies at broken edges of the lattice.

Fig. 3 illustrates the structure of attapulgite. The same fundamental grouping of ions exists here, but Si ions alternate first on one side of the oxygens, then on the other. If this diagram were continued, channels of fixed size would be illustrated where free water molecules are pictured. Particles of attapulgite are an agglomeration of minute needles.

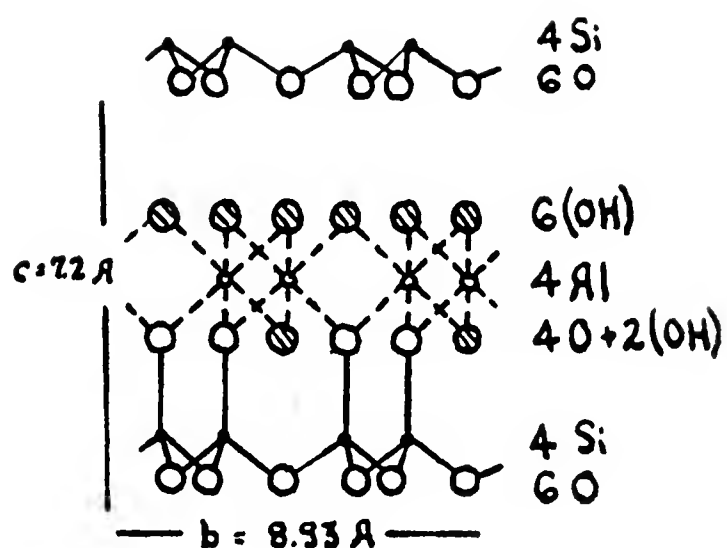


Figure 1



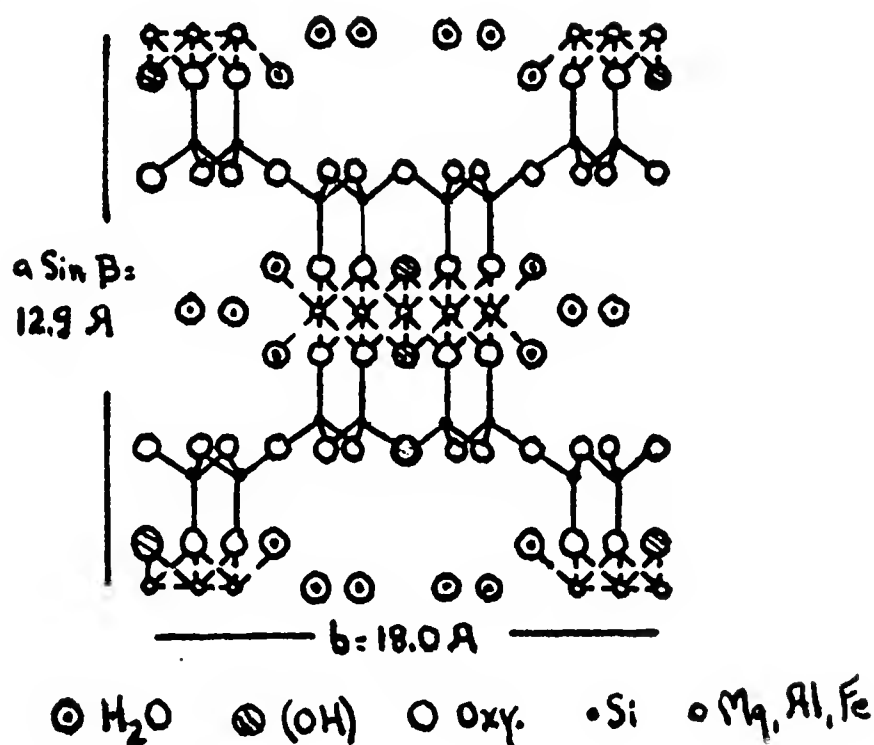
Pyrophyllite structure  
(after Pauling)

Figure 2



Kaolinite structure  
(after Gruner)

Figure 3



Attapulgite structure  
(after Bradley)

The needles have their long axis in a plane perpendicular to that of the diagram. Attapulgite is characterized by particles consisting of minute needles which have channels of fixed size. There is a small amount of isomorphic substitution in the lattice.

In addition to the above, one more structure, diatomite, will be considered. The name, diatomite or diatomaceous earth, is given to inorganic deposits of the shells of unicellular plants—the diatoms. Diatom skeletons are made up of an opaline form of silica and consist of patterns and partitions, plates and apertures of great variety and complexity. Since the

cell wall is only a few millionths of an inch thick, the internal structure is highly porous on a microscopic scale.

On the basis of the structural discussion above, characteristics and properties of diluents and carriers can be developed. The ones which will be discussed are particle shape, particle size, sorptive capacity, surface area, and cation exchange capacity. The fundamental structure of stacked layers found in pyrophyllite, talc, montmorillonoids, and kaolinites gives these materials particles which are flat plates. In general kaolinite materials used have a smaller particle size than either pyrophyllite or talc materials. Montmorillonoids characterized by expanding lattice structure may have large particles or under the proper conditions they may be dispersed to almost individual layers of colloidal dimensions. Attapulgite particles are an agglomeration of randomly-oriented minute needles. Celite particles are irregular in shape and have the form of diatom skeletons or parts thereof. The lack of penetration between the fundamental layers of talc, pyrophyllite, and kaolinite results in dense, non-porous particles, hence the sorptive capacity of these materials is not as high as attapulgite or diatomite. Kaolinite, however, has smaller particle size and generally exhibits higher sorptive capacity than do talc or pyrophyllite. Montmorillonoids will be of low sorptive capacity, except in cases where their large internal surface is available to polar compounds. Attapulgite and diatomite, because of the nature of their particles as pointed out above, have high sorptive capacity.

TABLE III—Surface Area and Cation Exchange Capacity of Five Typical Diluents and Carriers.

	Surface Area sq.m./g.	Cation Exchange Capacity m.e./100g.
Diluex A (attapulgite)	120-140	25-30
P.C. Bentonite (montmorillonoid)	≅ 20	60
Barden Clay (kaolinite)	23	5
Celite 209 (diatomite)	15-25	—
Pyrax ABB (pyrophyllite)	0.6	1

Table III lists the surface areas and cation exchange capacities for representatives of five of the groups which have been discussed. These values have been taken from Weidhaas and Brann (1955) and Mooney, Keenan, and Wood (1952). These materials have been chosen since they are typical of the groups they represent and these will be discussed later in connection with experiments on adsorption. The small needles of attapulgite give it a large surface area. The surface area of Panther Creek Bentonite is that of the external surface only. Total surface area including both internal and external is approximately 800 sq. m. per gram. The larger surface area of Barden Clay in comparison to Pyrax ABB reflects its smaller particle size. Large isomorphic substitution in montmorillonoids account for its high cation exchange capacity. Attapulgite has a smaller amount of isomorphic substitution with a resultant smaller cation exchange capacity. Lack of isomorphic substitution in the lattice of pyrophyllite, talc, and kaolinite gives these materials a low cation exchange capacity. In these materials the cation exchange capacity arises only from unsatisfied valencies at broken crystal edges. Kaolinite with its smaller particle size has a slightly larger cation exchange capacity.

From these general considerations of diluents and carriers, I would now like to consider some specific data on the interaction of some chlorinated insecticides and related compounds on the surfaces of typical diluents and carriers given by Weidhass (1955). While considering these data, the values given for surface area and cation exchange capacity should be remembered.

In these experiments the amount of adsorption of DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane), DDE, (1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene), methoxychlor (1,1,1-trichloro-2,2-bis (p-methoxyphenyl) ethane), methoxy-DDE (1,1-dichloro-2,2-bis (p-methoxyphenyl) ethylene), and DDA (2,2-di (p-chlorophenyl) acetic acid) on

the surface of five typical diluents and carriers was measured from organic solvent. The diluents and carriers used were those listed in Table III. The particular adsorbates used were chosen because they all have the same basic structure with differing functional groups. The adsorbents used were selected since they are representative of both commercially available products and widely differing mineral groups.

Adsorption studies were conducted under controlled laboratory conditions to give a maximum amount of adsorption. The diluents and carriers were oven-dried at 110°C for 48 hours and stored in a dessicator prior to testing. The adsorbates were purified. Adsorption was measured from hexane solutions for all of the adsorbates except DDA which was measured from benzene solution. Methoxy-DDE was also studied using benzene solutions so that all five adsorbates could be compared. A known weight of the diluent or carrier

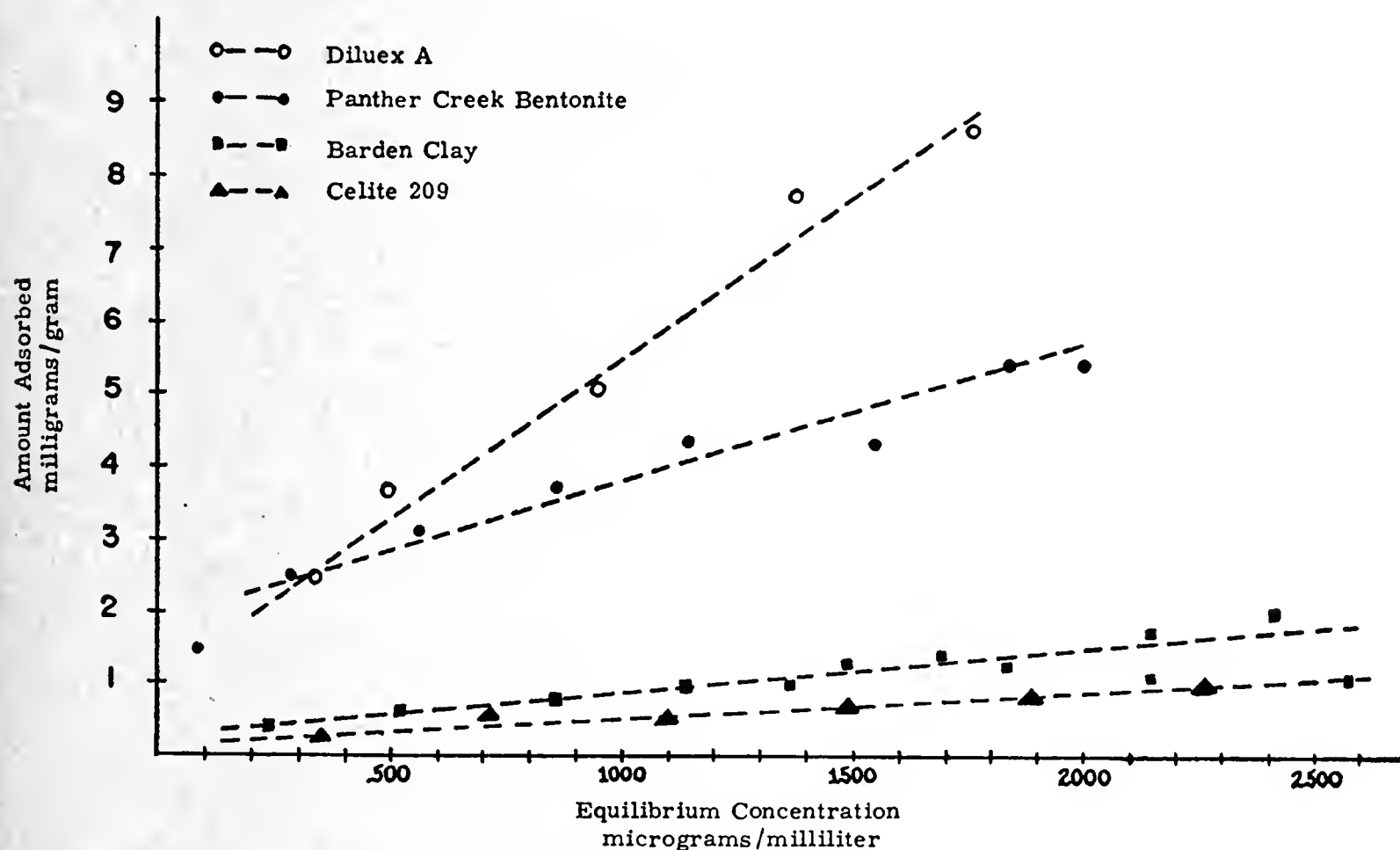


Figure 4. Adsorption of DDT on four types of diluents from hexane solutions.

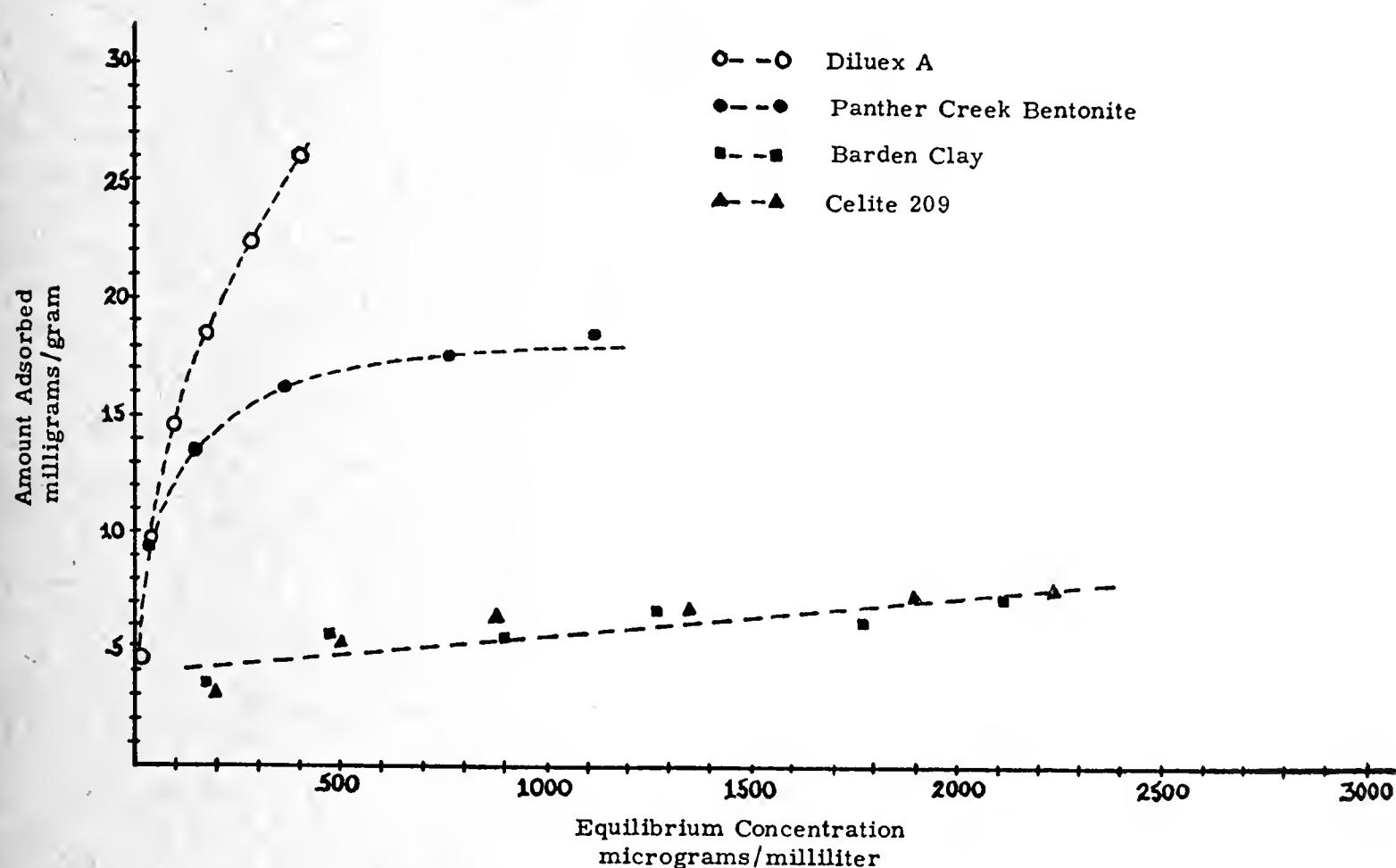


Figure 5. Adsorption of methoxy-DDE on four types of diluents from hexane solutions

was introduced into solutions of the adsorbates and the amount of adsorption was measured as the difference between the initial and final concentration of the adsorbate in solution.

Fig. 4 gives a summary of adsorption curves showing the amount of adsorption of DDT on four of the five diluents and carriers. In general the amount adsorbed is given in the following order: Diluex A > P. C. Bentonite > Barden Clay  $\approx$  Celite 209. There was no adsorption measured on Pyrax ABB so that this material would be ranked last in amount of adsorption. These results show that surface area has an effect on the amount of adsorption, but they show further that amount of adsorption does not correlate entirely with the surface area. This can be seen by comparing the adsorption and surface areas of P. C. Bentonite, Barden Clay, and Celite 209. From these studies it was concluded that only the external surface area of P. C. Bentonite was available for adsorption. Consequently these three materials have approximately the same surface areas, yet P. C. Bentonite adsorbs more than the other two materials.

In Fig. 5 the amount of methoxy-DDE adsorbed is plotted. Again there was no appreciable adsorption on Pyrax ABB. The amount of adsorption is much greater than was obtained for DDT, but the relative order of amount adsorbed is the same as given for DDT. Again a partial correlation with surface area is evident, except that P. C. Bentonite adsorbed more than Barden Clay or Celite 209. Furthermore, the adsorption curve of methoxy-DDE on P. C. Bentonite levels off. Calculation of the area per adsorbed molecule from the amount adsorbed in the levelling off region and the surface area give the area per adsorbed molecule as 74 square Angstroms. The approximate area of a molecule calculated from lattice dimensions given in the Structural Report of the International Union of Crystallography (1953) is 85 square Angstroms. This indicates the amount adsorbed is sufficient to cover the external area of P. C. Bentonite and constitutes a monomolecular layer. It is interesting to note that according to Grim (1954, page 134) the area per cation exchange position on the basal surfaces of montmorillonoids is approximately 80 square Angstroms.

TABLE IV—General Summary of Adsorption Data.

↑Diluex A	↑DDA
P. C. Bentonite	Methoxychlor
Barden Clay	Methoxy-DDE
Celite 209	DDT
Pyrax ABB	DDE

Table IV summarizes the studies of amount of adsorption. The arrows indicate increasing amount of adsorption. On a given diluent or carrier the adsorbates were adsorbed in the following order: DDA > methoxychlor  $\approx$  methoxy-DDE > DDT > DDE. A given adsorbate was adsorbed on the diluents and carriers in the following order: Diluex A > P. C. Bentonite > Barden Clay > or  $\approx$  Celite 209 > Pyrax ABB. The order of adsorption found for the adsorbates can be explained on the basis of the polarity of these compounds. Cassidy (1954, page 164) states as a general rule: "The more polar the substance the more extensive its adsorption at a polar surface, other things being equal." As pointed out earlier, the amount of adsorption on the five diluents and carriers cannot be explained entirely on the basis of their surface area. It is believed the presence of cation exchange sites adds polarity to the surface and increases the amount adsorbed. Calculations and comparisons of the area per adsorbed molecule for both DDT and methoxy-DDE showed the area per adsorbed molecule at a given equilibrium concentration to be: P. C. Bentonite < Diluex A < Barden Clay < or  $\approx$  Celite 209.

During the course of the studies on adsorption, reaction at the surface and catalytic change were observed. Of the five adsorbates studied this only was observed with methoxychlor and methoxy-DDE. It was only observed on three of the five diluents and carriers; P. C. Bentonite, Diluex A, and Barden Clay. Reaction at the surface was the formation of color on the surface upon adsorption. Methoxy-DDE and methoxychlor turned red on the surface of Diluex A and P. C. Bentonite. After standing for 24 hours the red color of metho-



xychlor on Diluex A turned green. Methoxy-DDE showed a pink color when adsorbed on Barden Clay. This pink changed to lavender upon standing. In addition to this color reaction, catalytic change was observed. The occurrence of catalytic change was established in two ways: incomplete recovery of methoxychlor and methoxy-DDE upon extraction with acetone and the presence of a yellow color in the acetone extract. Iler (1955) states that the acid nature of the aluminum ion in tetrahedral coordination has been correlated with the catalytic activity of silica-alumina catalysts. Since this same acidity of the aluminum ion in tetrahedral coordination is present in the cation exchange complexes of P. C. Bentonite, Diluex A, and Barden Clay, the catalytic change observed is believed to be correlated with the presence of cation exchange sites. Catalytic change occurred only on those materials with appreciable cation exchange capacity.

In summary I would like to state that diluents and carriers have been studied and developed on the basis of their fundamental structure as developed by workers in other fields. Some properties of importance to their use in insecticide application have been considered. In addition specific data on the interaction of some insecticides and related compounds on the surface of typical diluents and carriers has been presented and interpreted on the basis of the fundamental properties of the diluents and carriers. It is hoped that further study on basic information will lead to the replacement of some of the empirical testing proceedings with the ability to predict the right formulation for use.

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# Insecticide Application to Row and Field Crops

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## ABSTRACT

*Any new equipment for applying insecticides or fungicides to row or field crops should be adaptable to several problems, should give pest control equal or superior to that obtained with the generally accepted methods, and should offer an increase in the efficiency of the operation. Low gallonage, low pressure sprayers and dilute, semi-concentrate and concentrate air blast machines are now available for this purpose. These new type applicators offer one or more advantages over the dilute, high pressure sprayer. They may require less manpower, less water, less time, and possibly less toxicant for a given operation. They generally do not necessitate the use of cumbersome booms. In most cases, the distribution of the chemical over the crops is not as even as is desirable. Much of the success and acceptance of this new equipment may be attributed to the new highly effective chemicals, the speed of application, as well as a saving in the time, labor, and water required. The use of new equipment has not necessarily reduced the cost of spraying. The savings previously mentioned are often offset by the high initial investment and the cost of operation. Because of the complexity of this problem it will require the specialized skills and co-operation of chemists, engineers, plant pathologists, entomologists, and others to develop a reliable and practical solution. The answer is not the building of larger machines requiring more horse power and higher costs. Progress lies in the direction of a more efficient use of the power now being used through a better understanding and application of the factors involved in getting the chemicals from the tank to the plants.*

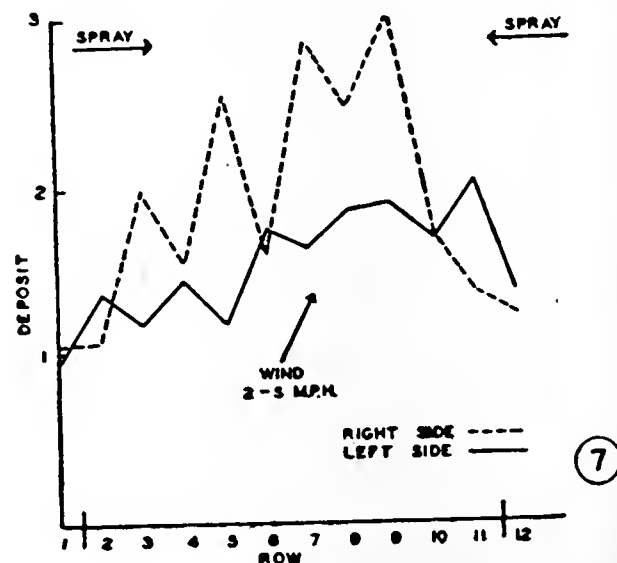
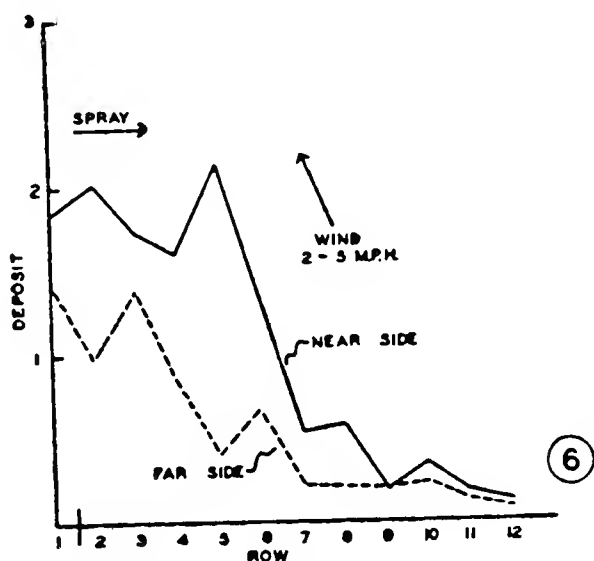
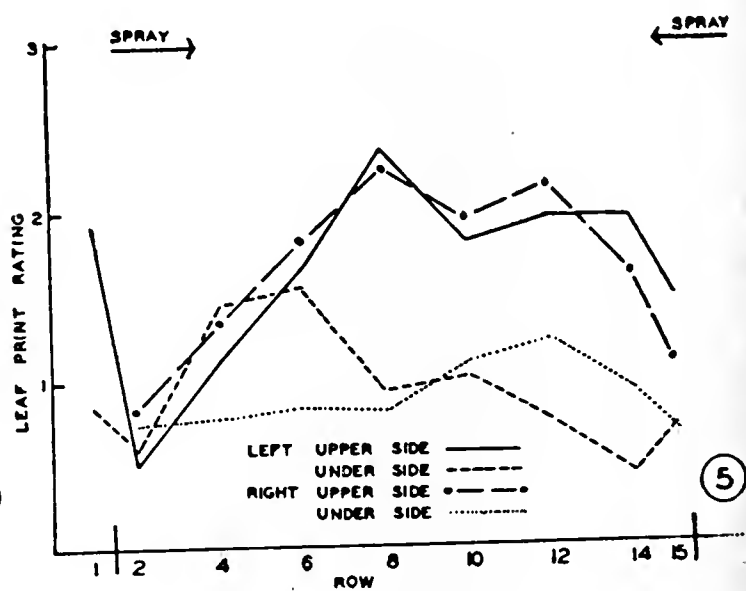
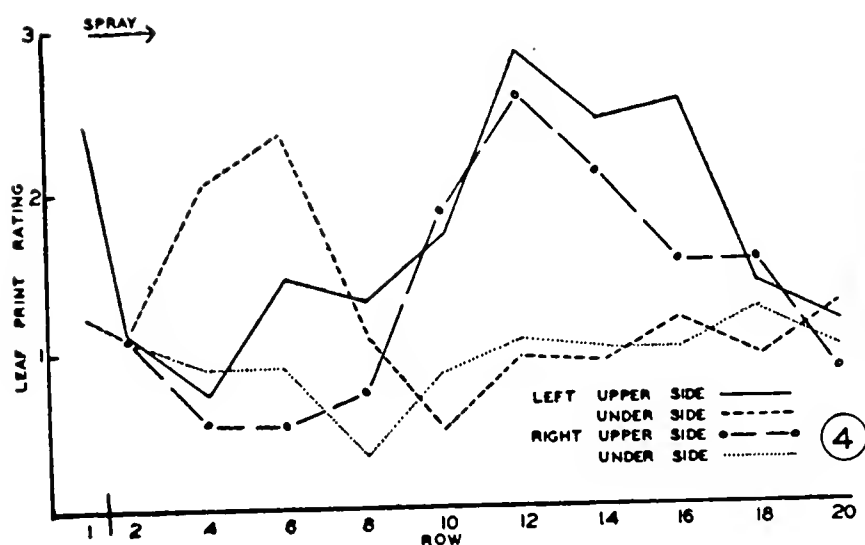
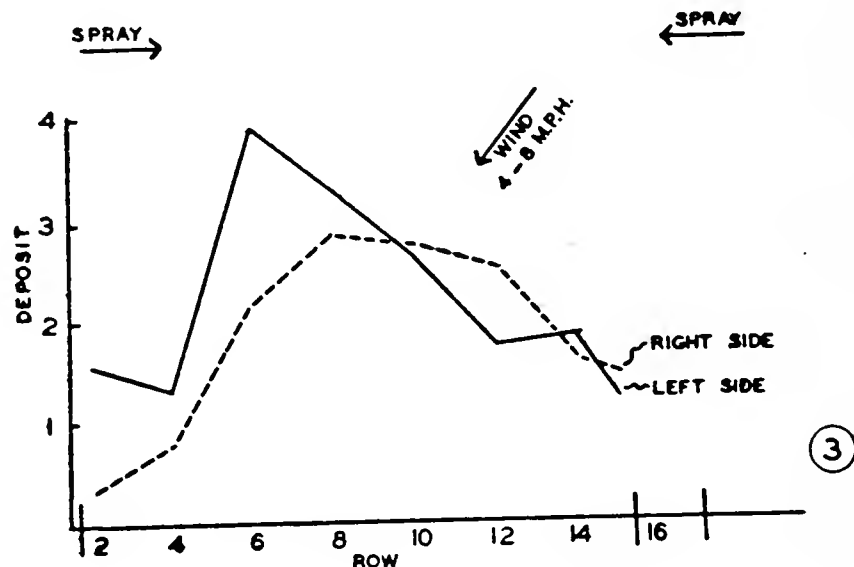
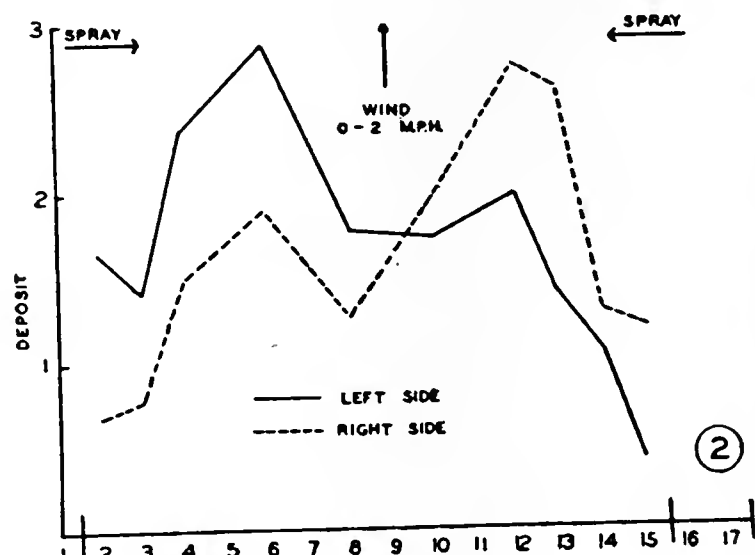
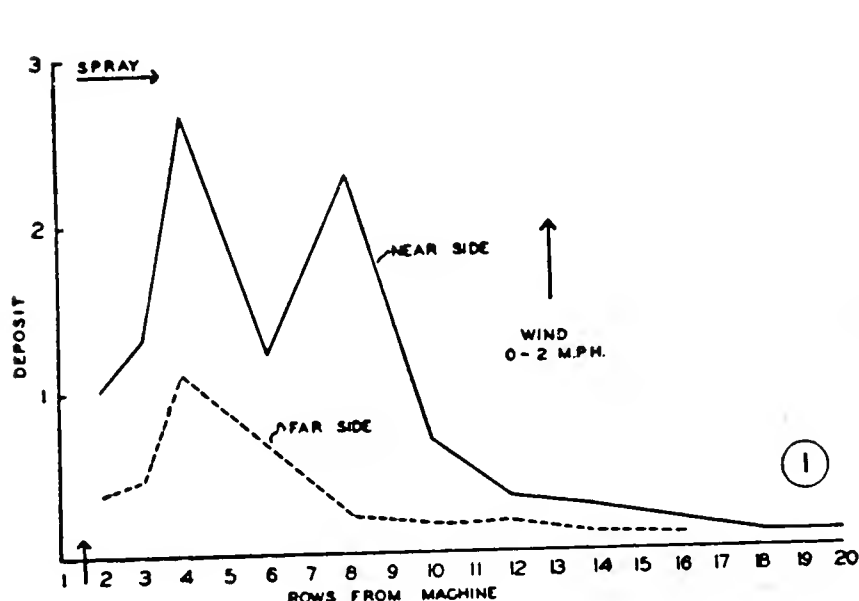
Ten years ago the big question in treating row or field crops for control of insects or disease was—what material should be used. Today we not only have to consider the material, but also the method of application and the type of equipment best suited to do an effective job of distributing the toxicant on the plants. The trend is to apply less liquid per acre over a wider swath through the use of low volume, low pressure sprayers as well as through the use of air-blast equipment applying insecticides either as dilute (100–200 gallons per acre), semi-concentrate (50–100 g.p.a.), or a concentrate (less than 25 g.p.a.). Inasmuch as air-blast machines, either semi-concentrate or concentrate, are becoming popular in spite of the many problems they present, this discussion will be limited to this type of equipment.

Great strides have been made in the development of air-blast equipment for the treatment of fruit crops. In fact, at the present, most of the deciduous fruit crops in the U.S.A. and Canada are being treated with air-blast equipment at some degree of concentration. Progress in the field of row crops has been much slower. Although most of the manufacturers of spraying equipment now offer one or more models of air-blast equipment for treating row crops, grower acceptance has been slow and adequate research data on the performance of these machines are not yet available. Some of the problems of treating row crops with air-blast are similar to those met in treating fruit trees, but others are unique and must be given special consideration. In tree spraying the bulk of the foliage is above the discharge mechanism of the machine and the foliage to be treated has considerable depth as well as breadth. In the case of row crops the outlets of the applicator are located entirely above the crop, the foliage is generally very dense and only one or two feet deep. When one tries to cover a wide swath of foliage, under these conditions all the adverse factors affecting the performance of spray droplets in an air stream seem to come into play.

Brown (1951), Potts (1946), Ripper (1955), Courshee (1954a, 1954b, 1955), Brann (1956) and others have discussed the problems of the volume and velocity of air-blast, droplet size, drift, and other factors as they relate to the deposit and distribution of spray droplets on crops. They stress the necessity of giving particular attention to these factors if a satisfactory deposit and distribution of the spray material is to be attained. However, as yet, most of the work has been theoretical or qualitative in nature and we do not have

<sup>1</sup>The author gratefully acknowledges: Drs. A. W. Avens and G. L. Mack, N.Y.S. Agricultural Experiment Station, Geneva, for making the chemical analyses; Professor W. W. Gunkel, Department of Agricultural Engineering, for co-operating on the engineering phases of the work; and Roy T. Cunningham and Glen Fleming, graduate assistants, for assisting with the laboratory and field work.

enough information on the interrelationship between the various factors and their effect on the deposit finally laid down on the crops under field conditions. On the other hand, growers of field and row crops, aware of the progress that has been made in the design of





fruit and tree spraying equipment, have demanded new and better equipment for treating their crops. In the U.S.A. at least, manufacturers have been quick to answer this demand. The machines offered are almost entirely adapted from fruit sprayers. The fan mounted on the rear delivers the air through outlets discharging at right angles to the direction of travel. Some of these machines deliver from one side, while others deliver from both sides simultaneously. The rate of application is usually between 50 and 100 g.p.a. Since the increasing use of such equipment makes it necessary to investigate their performance, rather extensive tests have been made with the Myers Air Blast, the Bean Air Crop, and the Iron Age Spray Blast machines. Less extensive tests have been made with the Buffalo Turbine and the Bean Speed Air. Because of the similarity of the swath laid down by all of these machines it seems advisable to discuss them as a group rather than as individual pieces of equipment. The data selected for discussion are presented as being typical of that obtained from this type of equipment and should not be considered as a criticism of any particular make.

A typical test consisted of operating the machine across a potato field just prior to full bloom while the plants were still upright. First a single swath was run to study the type and rate of deposit across the swath from the rows adjacent to the machine to those 60 ft. away. Then in another area of the same field two runs were made, 16 rows or 48 ft. apart, with the machine delivering the material in opposite directions in such a manner that the two swaths completely overlapped each other. Data were taken in the following manner. To determine the total deposit laid down, 100 leaf punches were taken from each side of each row half way between the ground and the top of the plants and analyzed for lead arsenate. Thirty whole leaves were taken from the same positions as the leaf punches and prints of the lead arsenate deposit were made in the laboratory using the technique described by Hamilton (1925). When these leaf prints were visually rated for the distribution and density of the deposit they presented a clear picture of the relative deposit on the upper and lower leaf surfaces.

Fig. 1 presents the results of the chemical analysis of the leaf punches taken from potatoes sprayed from one direction by the Iron Age Spray Blast machine (Fig. 8), applying 50 gallons of water and 3 pounds of lead arsenate per acre.

It will be noted that the far side of the rows received much less deposit than the near side; that the rows near the machine had much less deposit than those farther away and that the deposit fell off rapidly beyond the 8th or 9th row. It is obvious that one should not expect to get insect or disease control over a 60 ft. swath with this type of distribution.

When two 48 ft. swaths from the same machine were overlapped, or superimposed on each other from opposite directions the results of the chemical analyses (Fig. 2) were as might have been predicted from a study of Fig. 1. Although the over-all coverage was improved, the differences in deposit between the left and right side of the rows as well as the differences across the swath leaves much to be desired.

Fig. 3 shows the effect of a 4-8 m.p.h. wind on the deposit laid down by another machine of similar type, the Bean Air Crop (Fig. 9) applying 50 gallons of water and 3 pounds of lead arsenate per acre. Note that the curves are similar to those obtained from the Iron Age Machine but that the entire swath has been displaced by the wind.

Fig. 4 presents the results of a study of the leaf prints obtained from a single pass of the Bean Air Crop machine. It will be noted that the under side of the leaves on the near

Fig. 1. Lead arsenate deposit pattern across single swath obtained with Iron Age Spray Blast machine. Near side or far side indicates position on row from which samples were taken. Deposit refers to mg. lead arsenate per 100 leaf punches.

Fig. 2. Lead arsenate deposit across overlapped swath obtained with Iron Age Spray Blast machine. Perpendicular lines on X axis indicate wheel rows.

Fig. 3. Effect of wind on deposit pattern across overlapped swath applied with Bean Air Crop machine.

Fig. 4. Deposit pattern on upper and under side of leaves, right and left hand side of rows as determined by leaf prints. Single swath applied with Bean Air Crop machine.

Fig. 5. Leaf print study of overlapped swath applied with Bean Air Crop machine.

Fig. 6. Deposit pattern laid down on right hand side of Cornell Experimental Mist Concentrate Sprayer.

Fig. 7. Deposit pattern of overlapped swath laid down by Cornell Experimental Mist Concentrate Sprayer.

side of the rows adjacent to the machine are well covered, while the upper side of the leaves in the same areas are poorly covered. On the other hand, at 10 to 16 rows from the machine, the upper sides of the leaves are well covered while the under surfaces receive much less deposit.

Fig. 5 shows the results of the leaf print ratings of leaves taken from rows sprayed from two directions by the same machine. These are the same rows from which the chemical analysis data presented in Fig. 3 were taken. The result is what might in general be expected from reversing Fig. 4 and superimposing it upon itself. The coverage next to the machine is generally poor but is better on the under side of the leaves on the near side of the rows than it is in the center of the swath. On the other hand the coverage on the upper side of all leaves in the center of the swath is fairly good.

This irregular and somewhat unpredictable distribution of material on the plants is the result of trying to cover a 40 to 60 ft. horizontal swath with a single air stream. The volume and velocity of the air near the machine tends to tip the plants and turn the leaves so that most of the deposit is laid down on the under surfaces. At this point in the air stream the velocity is high enough so that both large and small droplets are deposited. Fifteen to twenty feet from the outlet the air blast has diminished so that it no longer effectively turns the leaves, the large droplets fall out on the upper leaf surfaces and the air velocity is too low for the impingement of the finer droplets. Work done on the determination of the droplet size produced by these machines has shown that they vary over a wide range. In fact almost any mass median diameter can be obtained depending on the part of the air stream that is sampled. Modifications in the air stream, nozzle arrangement, nozzle type and liquid pressure can change the amplitude of these curves, but within the limits of the work that has been done the general distribution pattern remains about the same.

At this time it might be interesting to discuss the performance of an experimental sprayer designed and built by the author and Professor W. W. Gunkel as a co-operative project between the Departments of Entomology and Agricultural Engineering at Cornell. This machine is very similar to, but approximately twice the size of, the smaller machine described by Brann and Gunkel (1953). These machines are unique in that the round outlets are mounted on a rotating turret (Fig. 10). The fan delivers to the turret approximately 10,000 cubic feet per minute of air which is discharged through the outlets at 150 m.p.h. during the forward 180° of rotation. The liquid is injected into the air stream during the forward 140° of rotation. Thus the swath pattern is a series of cycloidal curves laid down ahead of the machine as it proceeds across the field. The larger machine applies 5–20 g.p.a., operates at 2–4 m.p.h., and covers a swath 36 ft. wide. This new model was subjected to the same type of field tests as those already described.

Fig. 6 presents the deposit data obtained when the machine was driven once across a field of potatoes, applying 20 g.p.a. over a 36 ft. swath. Only the rows on the right hand side of the machine were sampled. It will be noted that the coverage on the near side of the rows is good for approximately 6 rows while that on the far side is considerably less, but follows the same general trend.

The distribution pattern shown in Fig. 7 is as might be expected from superimposing the deposit pattern of the previous run upon itself, except for the high deposit on the right hand side of the rows and particularly at the right hand side of the swath. This can be explained by the wind condition at the time the treatment was made. When the pass across the left hand side of the swath was made the following wind tended to lift the pattern and dissipate the finer droplets in the air. The right hand pass was made against the wind which tended to force the droplets down into the foliage.

This machine was designed with several fundamental points in mind.

1. The outlets are mounted high so that the air blast carried down into the crop at an angle, thus getting greater penetration of the foliage.
2. The air is delivered through round outlets in an attempt to maintain a high velocity over as great a distance as possible.
3. The high initial air velocity of 150 m.p.h. gives greater momentum to the larger droplets so that there is less tendency for them to settle out near the machine. The downward angle of delivery also helps with this.



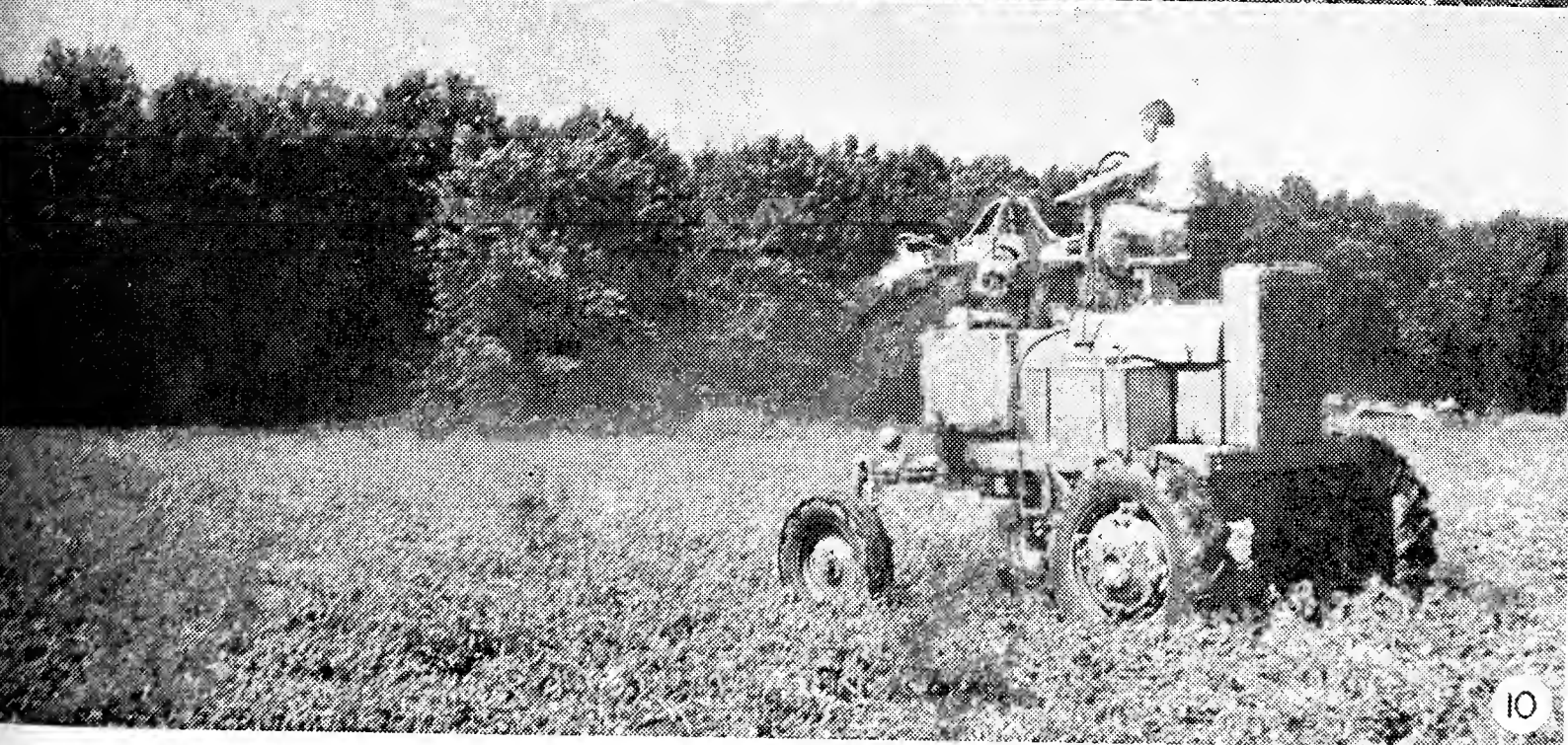
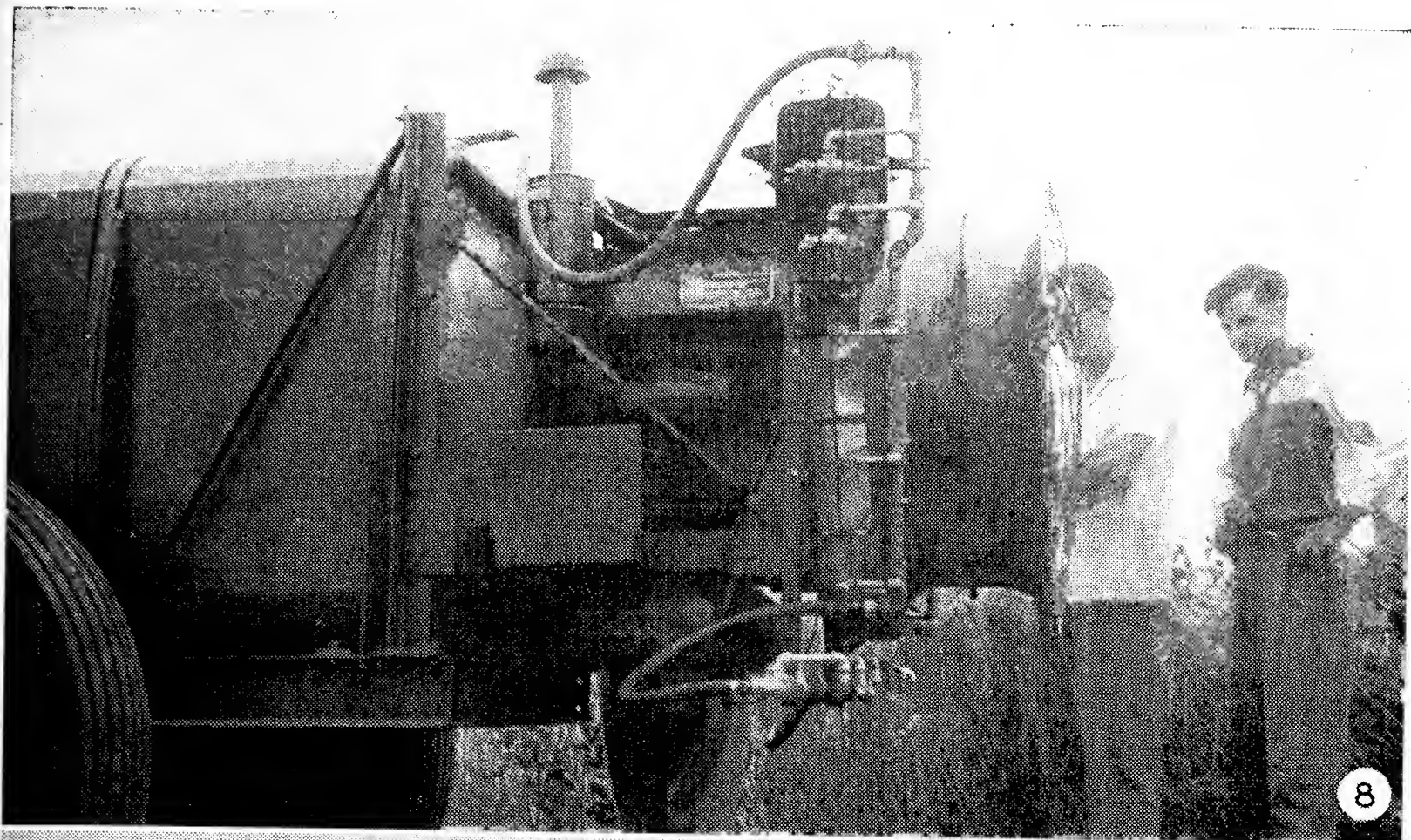


Fig. 8. Close-up of the Iron Age Spray Blast machine showing the modified keyhole type of air outlet.

Fig. 9. The Bean Air Crop machine in operation, applying 50 gallons per acre.

Fig. 10. The Cornell Experimental Mist Concentrate Sprayer applying 10 gallons per acre.

4. The successive cycloidal swaths laid down by the individual outlets agitate the plants and overlap one another to give a more even distribution particularly on the under sides of the leaves.
5. All plants are approximately the same distance from the outlets when sprayed.
6. Successive swaths across a field are applied from opposite directions so that all plants are hit from at least 2 sides.
7. Although the droplet size spectrum is fairly broad, it is possible to control and vary the mass medium diameter over a wide range.
8. The rows over which the machine passes are treated in approximately the same manner as are the other rows.

This machine is still in the experimental stage, but it does seem to offer possibilities for studying in detail a great many of the factors that are responsible for the deposition and distribution of spray materials on crops, particularly in the application of 20 gallons or less per acre.

Air-blast spraying, dilute, semi-concentrate or concentrate, of row crops is still relatively new. A great deal of research and developmental work is still to be done. It has been shown by many workers that the factors involved become much more critical as the concentration increases and the amount of liquid applied decreases. The farther plants are from the outlet the more difficult it is to obtain coverage. We need to discover, understand and learn to control more of the factors affecting the efficiency of this operation. We need to know more about the air blast, volume and velocity required for a certain job and how best to direct it to get adequate coverage. Droplet size becomes increasingly important as we go to high concentrations. We need to know how to control droplet size so as to produce a mist of as uniform droplets as is possible. Having done this we must determine what droplet size range is most satisfactory to use under given conditions. We know that temperature and humidity affect the deposition of mist concentrates. Some of our failures in insect or disease control may be attributed to not understanding the effect of these factors.

For an efficient operation we also need to know much more about amount and type of deposit that is required to control a given pest. In many cases a little toxicant in the right place will be much more effective than a lot of material scattered over the entire plant. The answers to these problems will not be easy to obtain but will require a great deal of research and co-operation between engineers, physicists, and chemists as well as entomologists and plant pathologists. We will also need the co-operation of the manufacturers.

Before concluding it might be worthwhile to make a few remarks regarding the present status of applying equipment. It is obvious that rapid development and ready acceptance of semi-concentrate air-blast spraying would not have been possible without the development of new and very effective chemicals for insect and disease control. In very many cases the machines as they are being used are giving a poor distribution of the toxicants over the crops. In analyzing samples from commercially sprayed crops we have found that the deposits vary up to as much as 6 to 1 from the bottom to top of a fruit tree, or across the swath in a potato field. However, the growers were getting control due to the effectiveness of the chemicals being used. When a grower is found to have applied 5 times as much deposit on one part of a swath as he has on another it is obvious that he is either not going to get control or that he is wasting 80 per cent of the material deposited. Since 60 per cent of the cost of spraying is in the materials, it is worthwhile to try to correct this distribution problem. It should be pointed out that new equipment has not necessarily cut the cost of spraying in all cases. In fact, the man power and time saved in the spraying operation has not always balanced the high initial investment plus the cost of operation and maintenance. On the other hand, the new equipment has aided considerably in many problems mainly through the speed of application. Growers can now cover their crops in a fraction of the time previously needed and can thereby time their applications so as to have a fresh cover of toxicant on the plants when it will be most effective.

And finally it is important to note that air-blast spraying today is in the same position that high pressure spraying was in the early 1940's. That is, we cannot go on indefinitely solving our problems by building bigger machines, delivering more and more air blast and



using more and more horse power. Progress lies in more efficient application of the power we are now using through a better understanding of the factors involved in getting the toxicant from the tank to the plants.

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# A Decade of Concentrate Spraying in Deciduous Orchards<sup>1</sup>

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## ABSTRACT

*Investigations on the mechanization of spraying in the orchards of British Columbia were begun at the Summerland Entomology Laboratory in 1946. Five years later most of the British Columbia fruit acreage was being sprayed with light, easily manoeuvrable, and relatively low-priced concentrate sprayers. The latest models are characterized by their low overall height, stainless steel or fibreglass-plastic tanks, high-pressure pumps, high-velocity blowers, and tungsten carbide nozzle components. The concentrate spraying procedure which, so far, has given best results in British Columbia requires from 50 to 75 imperial gallons of spray liquid per acre of mature trees. The use of non-ionic, water dispersible, surface-active preparations in summer concentrate spraying should soon become general. These preparations cause increased spray deposition in the tree tops, and eliminate objectionable spray-spotting in the lower branches; they have measurably improved the control of apple scab and the codling moth.*

## INTRODUCTION

In the 67-year history of the British Columbia tree fruit industry it is doubtful if any production method has contributed so much to the welfare of the fruit grower as concentrate spraying. The new method of applying pesticides reduced the cost of controlling insects and diseases to about half that of the high-volume, hand-gun spraying of earlier days, and transformed a foul and irksome job into a relatively simple, routine operation. Five years after concentrate spraying was officially approved it had been adopted by most of British Columbia's 3500 fruit growers.

The course of spraying methods in other fruit growing areas of North America indicates that the orchardists of British Columbia have mechanized their spraying operations with unusual speed and unanimity. There was little debate about the relative merits of high-volume and low-volume spraying. The reasons for the growers' ready acceptance of concentrate spraying were: (1) British Columbia's orchards are far removed from the principal fruit markets. High, fixed freight charges are a serious handicap; so every care has to be taken to keep the cost of production to a minimum. (2) Since the orchards average less than 10 acres most of them cannot support heavy capital investment, e.g., large and expensive air-blast sprayers. (3) Few of the orchard tractors are capable of hauling heavy spray equipment at slow speed in the predominantly hilly orchards. (4) The horticulturists of the area are averse to haulage of very heavy equipment through the orchards because of suspected ill effects of soil compaction. (5) Although many of the fruit trees of the area are fairly large—about 20 feet in height and 30 feet in diameter—they are pruned to allow adequate air circulation and entry of light; hence they are adapted to concentrate spraying with modestly powered machines. (6) Local manufacturers, who had no previous experience with spray applicators, and hence nothing to unlearn, sensed the significance of the new method of spraying, and quickly proceeded to build the type of equipment that had been officially approved, and that the growers were beginning to demand. (7) Official advisers were nearly unanimous on the merits of concentrate spraying.

## THE TERM CONCENTRATE SPRAYING

There is a good deal of confusion in the literature, and in the minds of fruit growers, as to what constitutes concentrate spraying. A simple way of dealing with the uncertain terminology is to group air-blast sprayers in two categories, high-volume sprayers, and low-volume or concentrate sprayers. A high-volume sprayer would be one which induces dripping of spray liquid from the trees; a low-volume, or concentrate sprayer, would be one which does not induce dripping. The suggestion is advanced because, perhaps the most significant feature of orchard spraying is the occurrence, or non-occurrence, of drip or "run-off". The character of a spray deposit alters radically after the spray droplets coalesce and begin to move downwards; from a discrete, or spotted deposit, a blotched, or

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filmed deposit takes form. Particularly in the control of fungus diseases the character of the deposit is important. Equally important, spray injury to foliage of fruit is commonly intensified when the drip-point is almost reached or passed. Assuming the trees are dry, the drip-point occurs in a mature British Columbia orchard when more than about 75 imperial gallons of spray liquid per acre are applied by an efficient concentrate sprayer. Inefficient machines, however, by heavily over-spraying the lower limbs, can induce dripping with considerably lower per-acre output. By the definition, such machines, though they may apply 60 gallons per acre, or even less, are not concentrate sprayers.

### HISTORY OF CONCENTRATE SPRAYING IN BRITISH COLUMBIA

The first Canadian work on concentrate spraying for deciduous orchards was done in 1946 (Marshall 1946). The following year spray concentrates were applied by five different types of mobile machines (Marshall and Miles, 1947). Although none of the machines were satisfactory the authors wrote, "There is little reason to doubt that conventional, high-pressure sprayers will shortly be supplanted by light, high-speed equipment". After listing what were considered the necessary features of a light, "automatic" sprayer, they mentioned that an experimental machine embodying these features was to be built at once. In a paper, presented to the Seventh Pacific Science Congress in 1949, they described the performance of the Okanagan experimental sprayer. It had proved successful and was serving as the prototype for commercial concentrate sprayers then being built in British Columbia (Marshall and Miles, 1953).

### TYPE OF SPRAYER

Most of the concentrate sprayers now being sold in British Columbia are produced by two manufacturers, both located in the fruit growing area, and both specializing in fixed air-vent machines. Since these manufacturers introduced the first machines in 1949 they have made many improvements in design. The weight of the "single-side" units has remained under one ton, but they have become lower, and streamlined in order to pass more readily beneath overhanging branches. Either two-, or four-wheel mounting is available, the latter being preferred because of lighter draft, lesser tendency to compact the soil, and more uniform spray application when travelling over rough ground. A troublesome problem in concentrate spraying has been corrosion of steel tanks, and consequent plugging of screens and nozzles. It has been overcome by fitting the machines with stainless steel or fibreglass-plastic tanks.

One of the British Columbia machines has a turbine-type blower producing a linear-flow airstream of 6500 to 7000 cubic feet per minute at a velocity of 105 to 120 miles per hour. The other has an axial fan which moves about 21,000 cubic feet of air per minute at about 90 miles per hour. When hauled at the rate of 1 mile per hour both machines have given adequate penetration of full-foliaged, well pruned trees up to 30 feet in diameter and 15 to 18 feet in height.

Because of the importance of adequate atomization piston type, high-pressure pumps are used on both the Canadian-built machines in preference to less expensive, low-pressure, centrifugal pumps or gear pumps. The hollow-cone, swirl-type spray nozzles are fitted with orifice discs and swirl plates made of sintered tungsten carbide (Marshall, 1951). One manufacturer has been fitting the pressure regulating valve with tungsten carbide inserts at the points of greatest wear. Although both machines are equipped with air-cooled engines, the performance of an experimental model fitted with a liquid-cooled, overhead-valve engine has been such as to suggest that liquid-cooled engines may shortly be optional.

To meet the requirements of some operators of large acreages "two-side" machines are being built by both of the British Columbia manufacturers. Unlike the "single-side" unit, which generally is operated somewhat beneath the overhanging branches, the two-side machine must travel mid-way between the two rows of trees. Consequently, unless the trees are of modest size, or exceptionally well pruned, adequate penetration of heavy foliage is more difficult to attain.

### EFFECTIVENESS OF CONCENTRATE SPRAYING

Using equal quantities of insecticide per acre experiments were undertaken at the Summerland Entomology Laboratory to compare spray deposits from thorough, high-



volume, hand spraying with deposits from concentrate spraying by a 25 horse-power turbine machine. The experiments involved both non-pruned, and well pruned apple trees 25 feet in diameter and 20 feet in height. These, in summary, were the findings: (1) In the dormant period oil deposits in the non-pruned trees were equal to those in the pruned trees, whether hand sprayed, or concentrate sprayed. (2) Concentrate spraying produced tree-top deposits of dormant oil four times as great as hand spraying with an equal quantity of oil per acre. (3) After the foliage had fully developed tree-top deposits from concentrate spraying were about 50 per cent less in non-pruned trees than in pruned trees; with hand spraying there was no difference. (4) Despite the reduction in tree-top deposits of DDT from concentrate spraying in non-pruned trees the deposits were still as heavy as from hand-spraying with an equal per acre quantity of DDT. It was concluded that concentrate spraying is a more efficient means of applying spray chemicals than hand spraying, and that it emphasizes the importance of good pruning (Waddell and McArthur, 1954).

Experimental work reported in 1953 (Marshall and Miles, 1953), later work (unpublished), and commercial results have shown that an efficient concentrate sprayer, well operated, provides as good control of the most important pests of British Columbia orchards as commercial, high-volume, hand-gun spraying. Among those pests are the European red mite, *Metatetranychus ulmi* Koch., the two-spotted mite, *Tetranychus telarius* (L.), the yellow mite, *Eotetranychus carpini borealis* (Ewing), the brown mite, *Bryobia praetiosa* Koch., the Pacific mite, *Eotetranychus pacificus* McG., the codling moth, *Carpocapsa pomonella* (L.), the San Jose scale, *Aspidiotus perniciosus* Comst., lygus bugs, the peach tree borer *Anarsia lineatella* Zell., the apple aphid, *Aphis pomi* DeG., the woolly apple aphid, *Eriosoma lanigerum* (Hausm.), the black cherry aphid, *Myzus cerasi* (F.), and the pear psylla, *Psylla pyricola* Foerst.

### FACTORS WHICH INFLUENCE THE EFFICIENCY OF CONCENTRATE SPRAYERS

In concentrate spraying the air stream, the blower scroll and air vent, the method of liquid atomization, the liquid manifold and that commonly underrated variable, the rate of travel, are important items. They are discussed in some detail in a general bulletin on experiments with a high-velocity concentrate sprayer in New York have indicated concentrate spraying for orchards (Marshall, 1958). Referring briefly to the last point, better codling moth control when the machine was moved at 1.5 miles per hour than at 2 miles per hour (Brann, 1950). In mist-spraying large trees in England, it has been claimed the rate of travel should not exceed 1.5 miles per hour (Pearch, 1955). Akesson, whose views are typical of many operators of heavy equipment, has written "In order to obtain the full benefits of blower sprayer operation it is necessary that the quantity of discharged air be sufficient to displace the air volume of the row of trees being passed." (Akesson, 1950).

When hauled at a rate of 1 mile per hour one type of British Columbia concentrate sprayer with a high-velocity, linear-flow air stream of only 7,000 cubic feet per minute gives satisfactory coverage of well pruned, full-foliaged apple trees up to 30 feet in diameter and 18 feet in height. If, to do so, it were necessary to displace all the air within such trees the machine would have to be moved past them at a speed of .025 mile per hour or less. It is, then, apparently unnecessary to displace all the air within a tree when applying air-assisted spray concentrates just as it is unnecessary to do so when applying high-volume spray liquids with a hand-gun. The air within the tree presumably becomes incorporated with that projected from the blower, and the mixture, highly turbulent by reason of the interference of branches, carries the spray droplets throughout the tree. It seems advisable, however, to travel slowly enough for the air stream to set the air in motion to the far side of the tree.

A considerable amount of experimental work has been done in British Columbia on the effect of rate of travel on spray deposition and pest control. The experiments have led to the conclusion that the 25 horse-power, one-side concentrate sprayers now being manufactured in the Province may be operated throughout the spraying season at a speed of 2 miles per hour among apple trees up to about 20 feet in diameter and 15 feet in height. Among large, well-pruned trees, 30 feet in diameter and 18 to 20 feet in height, a speed of 2 miles per hour seems adequate from dormant to pink-bud stage; later in the season a speed of 1 mile per hour is preferred.

## TYPE OF SPRAY COVERAGE

Early in the commercial use of concentrate sprayers in British Columbia it was observed that the growers were having more trouble controlling apple scab than the codling moth, or phytophagous mites—the opposite of the days of high-volume spraying. It was suspected that the trouble lay in the tendency of even the most efficient concentrate sprayers to over-spray surfaces directly opposed to the air stream, and to under-spray surfaces such as the side, or the stem-basin of the fruit.

Theoretically a uniform, film-like deposit should be more necessary in killing a motionless organism such as an apple scab spore than an actively moving insect or mite. In the first instance the toxicant must be placed close to, or in contact with the spore; a requirement which can be met fairly readily by the washing effect of high-volume spraying; in the second, the object of the spray unwittingly seeks out the toxicant, so that the deposition of the toxicant, even though relatively irregular, may be adequate for the job.

Since it did not seem likely that sufficiently uniform spray deposits could be achieved by mechanical means investigations were begun in 1951 to see what could be accomplished by chemicals, in particular by surface-active compounds or "surfactants". Five years later the use of surfactants<sup>3</sup> had become commercial practice in concentrate spraying in British Columbia.

In the operation of an efficient turbine-type concentrate sprayer D. B. Waddell of the Summerland Entomology Laboratory (unpublished work) found that only 2 per cent of the droplets were larger than the maximum wanted diameter of 100 microns. Those relatively few droplets, however, constituted 50 per cent of the volume of the spray liquid. Various authors have emphasized that since spray concentrates are applied in relatively small amounts, and there must be no coalescence of droplets and washing effect, as in high-volume spraying, the droplets must be very fine, and closely and uniformly spaced. That being so the detrimental effect to the spray coverage of the 2 per cent of large droplets must be considerable. But, if the surface activity of the spray liquid is so modified that all the droplets coalesce after impact to form a liquid film, then, providing the large droplets do not fall away from the air stream too rapidly, and providing there is no loss of toxicant from drip, the volume of spray material represented by the large droplets should be as effective in protecting foliage and fruit as an equal volume in the form of small droplets. The British Columbia investigations have shown that the efficiency of a concentrate spray deposit can indeed be measurably improved by the addition of a non-ionic, low-foaming, water dispersible surfactant. Presumably it functions somewhat as just discussed.

## FIELD EXPERIMENTS WITH SURFACTANTS

Since 1951 a considerable amount of experimentation has been done to assess the performance of surfactants in British Columbia. The results, in summary, are: (1) Surfactants have decidedly improved the control of apple scab, (Swales and Williams, 1956), and the codling moth *Carpocapsa pomonella* L. (2) They have not improved the control of phytophagous mites nor aphids. (3) They have not increased the effectiveness of dormant oil-lime sulphur mixture. (4) They have eliminated heavy spotting of sprayed surfaces close to the air-vent of the sprayer, and increased the spray coverage on inclined surfaces. (5) In trees up to 15 feet in diameter and 10 feet in height surfactants did not affect the quantity of spray deposit; but in large trees, from 20 to 30 feet in diameter and 18 to 20 feet in height, surfactants brought about an increase of 25 per cent or more in the deposits on the upper branches. (6) The concentration of surfactant necessary for concentrate spraying has proved to be from 10 to 40 times as great as recommended for high-volume spraying. (7) In the case of spray chemicals with a tendency to phytotoxicity surfactants have increased that tendency, but only in case there is drip. Consequently, surfactants are not suggested if the quantity of spray liquid is great enough, or the performance of the sprayer faulty enough, to result in drip. (8) With efficient equipment 50 gallons of spray concentrate per acre have proved adequate for controlling most insects and mites but, unless a surfactant was added, such a dosage did not give effective control of apple scab.

<sup>3</sup>Triton B. 1956. Rohm and Haas Co., Philadelphia, Pennsylvania, and Colloidal Spray Modifier, Colloidal Products Corp., San Francisco, California.

## SPRAY INJURY

Critics of concentrate spraying generally fault it on two counts; it is not sufficiently effective, and it is too likely to cause spray injury. The first criticism presumably arises from lack of information. It is not generally appreciated that many of the so-called concentrate sprayers, or conversion, or attachment units, now in use are incapable of applying concentrate sprays (less than 75 gallons per acre) safely or uniformly. These devices seem to have been manufactured and sold on the assumption that concentrate spraying merely means decreased output of spray liquid, and correspondingly increased concentration of spray chemical, and that almost any makeshift equipment capable of projecting air and liquid simultaneously can be called a concentrate sprayer. Fortunately those misconceptions are being corrected; but a certain amount of poor equipment is still being sold to growers who are more impressed with low cost than with efficiency.

As for spray injury there is no question that what has passed for concentrate spraying has sometimes caused severe damage to foliage and fruit. Spray chemicals that are prone to be injurious may be particularly so at high concentration. Certainly they should not be applied by machines with poor atomization, faulty distribution of spray liquid in the air-stream, or inadequate blowers. But the best of to-day's concentrate sprayers, properly operated, have proved capable of dealing with any of the spray chemicals in general use for high-volume spraying. There is another consideration; in view of the wide selection of acaricides, insecticides, and fungicides now available, it is questionable if it should be necessary to use any that are known to be hazardous to the tree, to the operator, or to the consumer of the fruit.

In England it was found that undiluted lime sulphur applied to apple trees as very fine droplets caused no injury; applied as large droplets it caused pronounced injury (Moore, 1948). The same result was obtained in British Columbia even when the large droplets were of dilute lime sulphur. It may seem surprising that lime sulphur, that excellent but caustic insecticide-acaricide-fungicide of long standing, should be more likely to cause injury to fruit or foliage if applied highly diluted than highly concentrated. Evidently it is largely, if not entirely, a matter of the rate of drying of the spray deposit; the sooner the spray deposit dries the less the likelihood of lime sulphur injury. A tree drenched by high-volume spraying takes considerably longer to dry than one that has been sprayed by the fine mist of a concentrate machine.

## SOIL CONTAMINATION BY SPRAY CHEMICALS

In the spraying of orchards a problem not always given the consideration it merits is contamination of the soil by spray chemicals. Experiments carried out by J. M. McArthur and his associates in the Okanagan Valley of British Columbia (unpublished data) have shown that concentrate spraying has a practical bearing on that problem. Over two sprayed areas of a mature apple orchard about 2.5 times as much DDT lodged on the cover crop where high-volume sprayed as where concentrate sprayed, although not quite one-third more DDT was applied by the high-volume procedure. Doubtless the main reason for the difference in amounts of spray chemical reaching the cover crop, or orchard floor is the absence of drip from concentrate application.

## ASSESSMENT OF CONCENTRATE SPRAYERS

The development of concentrate spraying as a practical proposition for British Columbia fruit growers necessitated a rapid means of screening out inferior machines or modifications of existing machines. Eventually a technique was evolved, largely by D. B. Waddell and J. M. McArthur, which, while admittedly laborious, has nevertheless, proved most useful. It has been described in some detail (Marshall, 1958) but a few words may be said about it here.

A machine under test is driven at a 30 degree angle past a vertical frame 30 feet high and 30 feet long, to which are attached targets in 30 different fixed positions. The targets are in pairs, one a white card the other a glass slide. The machine is charged with a water solution of the dye Rhodamine B in known concentration. The mass of the spray deposit at the various positions is determined by washing the glass slides with distilled water and analyzing the solution by a spectrophotometer. The size of the spray droplets is determined by examining the white cards under a stereoscopic microscope. Uniformity of coverage is

assessed by mounting strips of the white target cards on black album paper, the arrangement corresponding to that on the spray frame.

Machines, or modifications, that are obviously inferior to the reference machine, a 25 horse-power, high-pressure, single-side, turbine-type unit, are eliminated without further ado. Machines apparently as good as the reference machine, or better, are given orchard trials for chemical and biological analysis.

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# Distribution of Insecticides from Fixed-Wing Aircraft and Ground Power Equipment with Special Reference to Physical and Biological Factors<sup>1</sup>

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## ABSTRACT

*The studies here reported were obtained by a team of Entomologists and Engineers of the Agricultural Research Service, United States Department of Agriculture, at Forest Grove, Oregon.*

*The distribution of liquid and solid particles from aircraft is basically effected by the aerodynamic forces comprising the propeller slipstream and the wingtip vortices. The resulting distribution pattern is modified by secondary eddies resulting from drag components such as landing gear and dust distributors. Within limits, the swath width is directly related to boom length (or width of the distributor span) and flight elevation and inversely related to particle size. The latter is also inversely related to uniformity of deposit and directly related to drift.*

*Aerial applications have been most effective against active insects whose own wanderings insure insecticidal contact, or against exposed semi-sedentary insects such as the pea aphid. They have largely failed where deep canopy penetration and under-leaf coverage is required, as with the green peach aphid on potatoes.*

*Specially designed ground spray and dust applicators have been developed for controlling the pea aphid on peas and the green peach aphid on potatoes. The essential principle in both cases was to deliver the insecticide in required quantities as directly as possible to the favored microhabitat of the pest. Where under-leaf coverage was required, near ground level nozzle placement proved essential and, in the case of sprays, coverage was improved through the use of fine atomization and an auxiliary air blast.*

## INTRODUCTION

This discussion is developed from information obtained by the Entomology and Agricultural Engineering Research Branches of the Agricultural Research Service of the United States Department of Agriculture at Forest Grove, Oregon from a jointly conducted cooperative project for the development and improvement of aerial and ground equipment used to apply insecticides and for the development of improved methods of application. The studies were initiated in 1947-48 and have been continued since that date as a joint undertaking in cooperation with the State Agricultural Experiment Stations of Oregon, Washington and Idaho.

In the practical use of any agricultural chemical—insecticidal or otherwise—three essential elements are involved: the chemical itself; the crop, land, disease or pest to be treated; and the mechanism by which the material and subjects are brought into effective contact. Only two of these factors are subject to direct control, these being first, the material and second, its application.

The effectiveness of modern agricultural chemicals is, with few exceptions, of a much higher order of efficiency than the available means of application. Furthermore, it seems probable, in spite of foreseeable progress, that this disparity must always exist, especially where large-scale applications of contact insecticides to widely scattered insect populations are involved. In such cases, even with insecticides possessing great residual properties, most of the material is irrevocably lost through lodging on non-infested surfaces or in crevices where the insect does not occur or is unlikely to wander. In order to reduce these material losses to a minimum, it is of importance that any applying device be so designed as to deposit the insecticide as efficiently as possible in the actual microhabitat of the insect in question.

This objective may usually be realized to a large extent in designing and using ground equipment. In contrast, it may be virtually impossible with airborne equipment. In such

<sup>1</sup>Much of this information has been published as follows: Chamberlin, Joseph C., and V. D. Young. 1956. Insecticide application with aircraft and ground rigs. *Farm Chemicals*, 119(12): 50-53, illust.

cases the only feasible objective becomes to distribute the toxic material as evenly as possible across the treated swath with the purpose of avoiding wastefully high deposits in certain areas and subminimal amounts in others. Within these limits, insecticides must generally be applied from the air at substantially higher than minimal rates in order to increase the chance lodging of adequate amounts within a desired microhabitat.

With these considerations in mind, it is purposed to discuss in general terms some of the principles surrounding the application of insecticides from fixed-wing aircraft with special reference to the control of such insects as the pea weevil and pea aphid on peas and the green peach aphid on potatoes, as well as the use of an experimental ground dust and spray applicator specially designed for control of the green peach aphid on potatoes.

### DISTRIBUTION OF INSECTICIDES FROM FIXED-WING AIRCRAFT

Our studies have confirmed the idea that the distribution of insecticides from fixed-wing aircraft is essentially a function of the aerodynamic forces or air streams set in motion by the flight of the aeroplane itself. The trajectory and ultimate place of deposit of any individual insecticidal particle, whether liquid or solid, is therefore the resultant of the forces imparted to such particle by the forward speed of the aeroplane, by the air streams coincidentally generated, and by the action of gravity. Incidentally this results in a differential sorting of particles by volume and weight so that coarser and heavier (i.e. less buoyant) particles tend to drop out of the supporting air currents and to reach the ground closer to the point of origin than finer particles which remain suspended longer and follow a more intricate trajectory in arriving at a final, and often more distant, point of rest.

The air currents generated by an airplane in flight constitute an expanding wake of three vortices produced by the propeller slipstream and the two wingtips. These are accompanied or modified by secondary, parasitic eddies caused by landing gear, spray and dust distributors, and other attachments. The wingtip vortices comprise the most visible and spectacular part of the overall spray curtain and are set in motion by the inboard flow of air from the high pressure area beneath the wings, around the wingtips and into the temporary low pressure area above the wings. This spiraling flow carries the more buoyant spray and dust particles well out beyond the wingtips and back again, perhaps for several revolutions, but never outboard beyond the rather definite limits set by the speed of the airplane, by the nature of the air foil, and by distortions, resulting from impact and flattening of the vortices against the ground at low flight elevations. The propeller slipstream tends to "trap" spray or dust materials introduced therein. Unless modified by secondarily induced, parasitic eddies, this prevents such materials being drawn outboard into the wingtip vortices. It also tends to deposit its load distinctly left of the center of the line of flight in a very erratic and non-predictable pattern as a result of the counter clockwise rotation of the propeller. This is especially evident when the sampling area is small and when the flight level is low. In the midwing zone between the periphery of the propeller slipstream and the wingtip vortices there is a short space in which the discharge is relatively little influenced by either vortex. This is evident only in relatively slow and low-powered aircraft, to which category most present-day agricultural airplanes belong. In larger, faster, and more powerful aircraft, this neutral zone becomes vanishingly small and may be disregarded. In such cases spray or dust particles introduced into the tension zone between propeller and wingtip vortices will be caught in both and thereby distributed over the entire potential swath. With slower aircraft, booms or spreaders must extend slightly, at least, beyond the neutral midwing zone and well into the wingtip vortex if maximum swath widths are to be obtained. Since the aerodynamic forces with which we are dealing are extremely powerful, and uncontrollable except by actual aircraft design, about all that can be done is to introduce the solid or liquid particles, in proper proportion, to those points or zones where the major air currents discussed can take over and by their own characteristics determine the distribution pattern.

### FACTORS IN SPRAY DISTRIBUTION PATTERNS

The foregoing factors have a special bearing on the problem of specific nozzle spacings for spray applications. Thus with fixed-wing aircraft having an even distribution of nozzles across a full-span underwing boom, the spray deposit rate across the line of flight (or swath) is most streaked or variable for coarsely atomized sprays and for lowest possible flight levels (i.e. with the landing wheels actually marking the treated crop). This variability



becomes progressively less as the applying level increases until at 15 to 25 feet or more it has been reduced pretty much to a minimum. Thus, for high-level applications for the control of forest insects, mosquitoes, grasshoppers, and so on, no special nozzle arrangement (other than the usual even spacing) may be required. The same is also true even at low levels where *very finely* atomized sprays are employed. Indications are that special, corrective (non-symmetrical) nozzle spacings show no particular advantages or disadvantages after the applying level reaches 15 to 25 feet or more. Below this level, empirically determined, compensated nozzle arrangements have shown substantial advantages with bi-

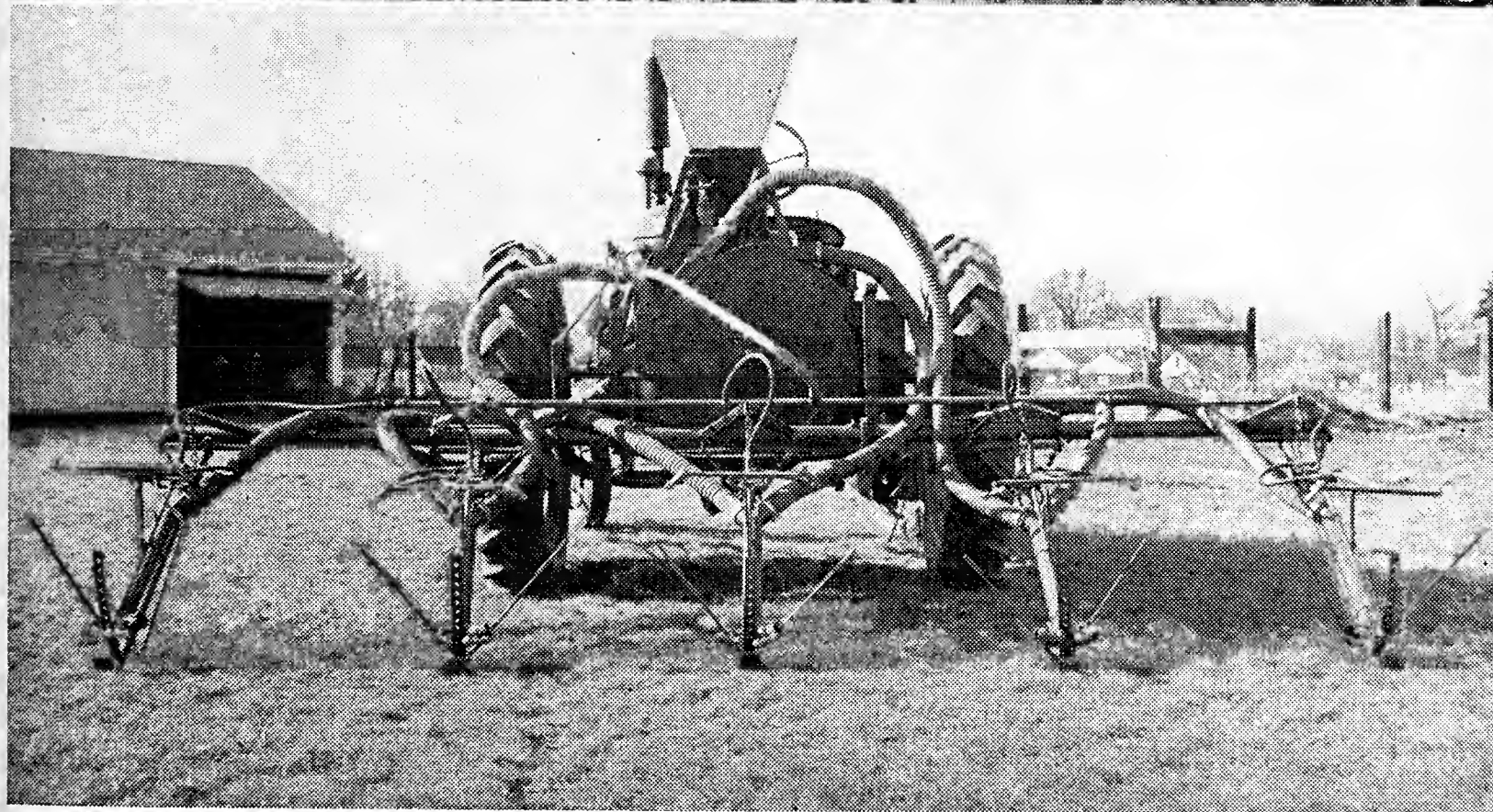
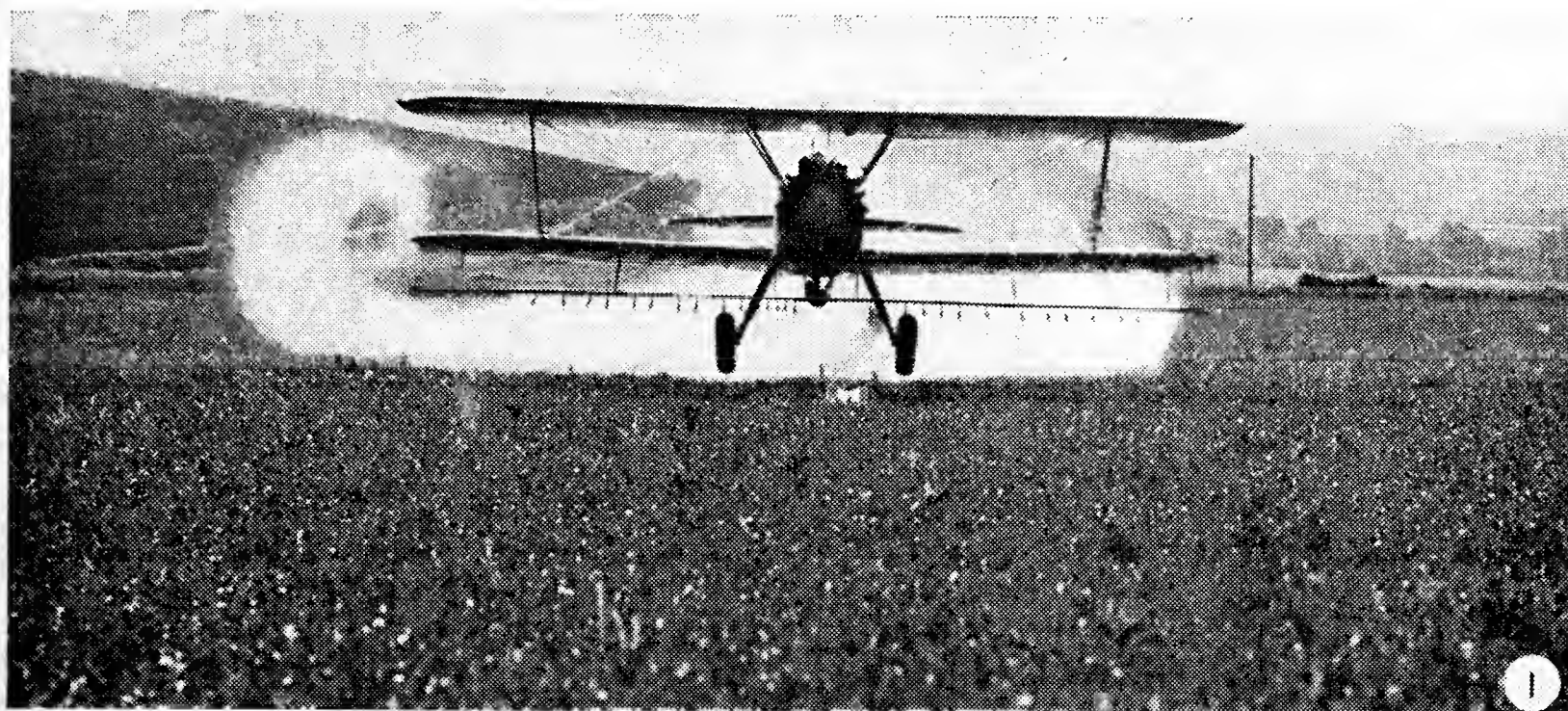


Fig. 1. Low level airplane spray application for control of the pea aphid near Walla Walla, Washington. Note the compensated nozzle spacing for insuring most even possible swath coverage. Also note the typical wing tip and propeller vortices.

Fig. 2. Combination row crop, duster-sprayer ground applicator with free floating trailing booms specially designed and constructed for control of the green peach aphid on hilled potatoes.

planes and low-wing monoplanes where medium or coarse sprays are employed. The need for compensatory nozzle spacings is greatest within the diameter of the circle described by the rotating propeller. With low-powered, high-winged, monoplane aircraft, however, the need for compensatory nozzle spacing is slight, even within the slipstream zone.

Equipment for the discharge of insecticides from aircraft varies with the formulations employed. A spray distributor comprises essentially a tank, pump, power source and boom, the latter provided with more or less evenly spaced openings or nozzles for discharge of spray. The boom may be suspended or hung below or behind the wings, or concealed within

the wing. The only critical feature in the system is that all parts—valves, pump, boom and other plumbing—be of sufficient capacity to discharge the maximum required amounts of spray at points where it may be picked up and distributed in proper proportion by the air currents previously considered. The pump itself may be driven by hydraulic motors powered by the engine itself; more rarely by electric motors or small stationary gas engines, or, most usually, by small windmilling propellers or fans mounted in the slipstream. For an ordinary small plane, the power required is from 4 to 7 or 8 horsepower depending on the pressure and the discharge rate. The pump should be capable of delivering between 25 and 100 gallons of spray per minute for most operations.

### FACTORS IN THE DISTRIBUTION OF DUSTS AND GRANULAR MATERIALS

The aerial distribution of dusts or granular materials is, of course, determined by the same forces governing the distribution of sprays. Thus far the dispensation of non-liquid insecticides has been accomplished by gravity flow of the material (with or without mechanical or pneumatic agitation) from a centrally mounted hopper into the throat of an air-scoop mounted within the propeller slipstream. This scoop is provided with diverging ducts extending astern toward the trailing edge of the wing and with the outboard ducts discharging their loads into, or rarely slightly beyond, the periphery of the propeller vortex. This dust distributor or "venturi" induces a secondary vortex at either outboard corner which distorts the normal symmetry of the slipstream and carries the dust or granules farther outboard than would be otherwise possible. Thus with an N3N or Stearman biplane and a 3-1/2 to 5-foot wide distributor, a 30-35 footswath is actually obtainable at low flight levels (3 to 6 or 8 feet) because of this fact instead of approximately 15 to 20 feet to be expected from the propeller slipstream alone. If the usual dust distributor is constructed so as to extend the outboard ducts *beyond* the effects of the propeller slipstream (i.e. to a distance outboard substantially greater than the radius of the propeller), a much wider swath is at once obtainable—approximating that obtained with sprays from a full underwing boom—that is about 55 to 60 feet at a 6-8 foot flight level. However, such wide distributors, as commonly constructed, cause a tremendous drag necessitating either dangerously low flight speeds or the use of excessively high engine power. By thinning out and streamlining such a distributor, however, thus giving it something of the character of a swept-back miniature wing, an experimental distributor with an overall width of more than 14 feet has been constructed and tested. This "wing-like" distributor actually produces some lift and has so little drag that the speed of the airplane is scarcely impaired.

### INSECTICIDAL EFFICIENCY OF AERIAL APPLICATIONS

Our studies along this line have been limited to tests with a relatively small number of insect pests, principally of peas, potatoes, beans, alfalfa, and a few other miscellaneous crops. Notwithstanding the limited nature of our experience, these and other studies suffice to demonstrate that, granted the availability of a suitable insecticide, aerial applications are most effective: (1st), against active insects such as the pea weevil, the beet leafhopper or cucumber beetle, the activities or wanderings of which (especially in the case of the residual-type applications) insure effective contact, or (2nd) against *exposed*, sedentary insects such as the pea aphid, infestations of which are greatest on the tender terminal growth. They have largely failed where deep canopy penetration and good underleaf coverage (especially of the lower leaves) are required as with the green peach aphid on potatoes, the black bean aphid on pole beans, or the two-spotted spider mite on hops.

It is to be recognized that relatively little direct control can be maintained over insecticidal dusts or sprays applied by aircraft once the discharge has been effected. They are literally carried to their destination on the wings of the wind and are therefore subject to its every caprice. Uncontrollable drift on both horizontal and rising air currents is therefore an especially important limiting factor in aerial application. Low flight elevations; minimal wind velocities; maximum effective particle sizes (dry or liquid); and cool ground surface temperatures, with consequent lack of rising air currents are among the most important indirect methods of obtaining satisfactory insecticidal deposits. The use of extremely low flight levels and near-stalling plane speeds to obtain maximum agitation of foliage and consequent better coverage is not only a dangerous practice but a largely ineffective one



as well, principally because such flights do not actually displace the "dead air" beneath the foliage canopy. As a result the insecticide-laden air currents are unable effectively to penetrate to the desired places and hence deposits are largely dependent upon a purely gravitational fall-out. In other words, the superficial agitation of the exposed leaves by the slipstream does not significantly improve foliage penetration or coverage of the under surfaces of leaves except possibly at the top canopy level. Where canopy penetration but not coincident *underleaf* coverage is the principal requisite, sprays have been found to give better results than dusts. This is almost surely because of the greater mass and inertia of the spray droplets as compared to the very fine and buoyant dust particles.

Since the insecticide-laden air cannot be specially diverted to desired portions of the treated swath, weeds, non-hosts, and bare ground are treated equally with the infested crop plants, resulting in undue wastage of material especially in the case of row crops. Consequently, as earlier noted, for any given insecticide, applications with aircraft must nearly always be substantially higher than with well-designed ground equipment.

### CONSIDERATIONS IN THE DESIGN OF SPECIALIZED GROUND APPLICATING EQUIPMENT

This highly diversified field is largely beyond the scope of the present discussion. Unlike aircraft, surface equipment may be designed with the special characteristics of the crop as well as a specific insect pest and its microhabitat in mind.

As an example, we experienced outright failure to control the green peach aphid on potatoes with some of our most potent insecticides applied with aircraft at highly excessive rates. Thus malathion spray at 9.6 (!) lbs. of active ingredient per acre, gave only poor control (about 70%), while parathion dust at a net rate of 1.1 lbs. per acre and diazinon dust at 2.2 lbs. active ingredient per acre failed to give any control whatever. However, with a specially constructed experimental ground dusting and spraying unit built with the cultural conditions and growth habit of the potato, and the specific location of maximum aphid infestations in mind, we obtained excellent control with the same materials at half or less the rates which failed completely with aerial applications.

The essential consideration in the design of this equipment was to direct the dust or spray as the case might be, laterally and upward from a point only two or three inches above the soil surface of the furrows between the potato hills so that the initial impact of the insecticide is to the undersurfaces of the leaves on the lower story of the plant canopy or to the actual microhabitat of the insect in question. With this type of ground application, the function of the plant canopy is that of a tent confining and restraining the dissipation of the insecticide. This is in strong contrast to its role in aerial applications where it serves rather as a protective umbrella to the insect in question.

The equipment itself comprised five free-floating, trailing booms each provided with a skid supporting the necessary spray nozzles and dust orifices. The entire unit was mounted on a 4-wheel tractor. Except in actual operation the trailing booms are elevated with a hydraulic lift. With this equipment drift is reduced to an absolute minimum although, even so, a short canvas trailer proved advantageous in the case of dust applications when the vines were relatively small. When vines were large, however, the canvas trailer proved unnecessary, and even in winds of 8-10 miles per hour or more no significant loss of dust or spray from drift was observed.

### CONCLUSION

In conclusion, it may be pointed out that future applying equipment, whether ground or airborne, may be expected to show not only increased improvements but increased diversification as well, in response to specialized needs. No informed worker or thinker expects the ultimate development of a "universal insecticide" which will destroy all insects in all situations. Neither should he expect a single universally useful method or mechanism for the application of such materials.

Contrary to the opinion of some operators, aerial and ground applying methods are no more mutually antagonistic than is the saw and the hammer. Rather they are both useful and complementary in nature. It is the duty of future workers to explore and develop the potentialities and limitations of each with respect to individual requirements. In other words, progress in this field, as in any other, may be expected to result in increasing

specialization and differentiation of our applying tools, whether ground or airborne, in response to specific needs. Finally, since the nature and physical properties of an insecticidal formulation are basic to the design of an efficient dispersal mechanism, the manufacturer and formulator of insecticides should work even more closely than in the past with the engineer, the entomologist, and the applicator if the requirements of agriculture are to be most advantageously served.

# High Altitude Application of Insecticides from Aircraft

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## ABSTRACT

*In many types of large-scale insect control operations, terrain and vegetative cover of terrain dictate the use of high payload type aircraft for economic reasons, flying at heights of more than 50 feet above ground or tree-top level for safety. Some principles of application involved are similar to those applicable to the use of low-flying light aircraft; other principles are drastically different. These principles, as elucidated during the development of insecticide dispersal equipment for the (C-47) Dakota aircraft, including the effect of location of the spraying devices on the aircraft on the pattern of ground deposits, the effect of meteorological conditions on spray coverage, and the efficiency of various atomization devices, are discussed. The physical properties of sprays as related to the efficacy in penetration of foliage and deposit coverage, are discussed briefly and emphasized as prerequisites to any application of dry granular carriers for insecticides from high capacity aircraft.*





# The Comparative Assessment of Locust Control Methods

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## ABSTRACT

*For the Desert Locust, of which the numbers have been shown to be at times large compared with the control resources available, and against which the main control effort is made far outside the crop areas concerned, on locust populations ranging in density from several thousands to less than one individual per square metre, the most appropriate criterion for comparative assessment is considered to be locusts killed per unit of insecticide, leading in turn to estimates of kill per unit of overall expenditure, and making possible comparisons not only between different control methods in the field but also between field and laboratory results.*

*Examples are presented of field assessments providing such data in respect of baiting and spraying operations against the nymphal stages (hoppers), and of contact spraying against adult swarms, both settled and in flight.*

*The data so far available are utilised to illustrate the possibilities of an objective approach to the problem of comparative economics. As an example, it is shown that, in extensive aircraft spraying operations against swarms in East Africa during 1954 and 1955, kills are likely to have varied between limits corresponding to costs equivalent (in terms of small-scale bait assessments) at worst to some of the poorer (though not the poorest) of the hopper baiting results, and at best fully comparable with those for the best experimental bait kill yet recorded.*

The development of newer methods of locust control, some complementary and some potentially alternative to established methods, is posing the increasingly acute problem of the comparative assessment of old and new methods, on a common quantitative basis. The assessment should be capable of providing an objective criterion for the allocation of resources between the different methods currently available. The initial application of operational research methods to such problems of locust control was due to Gunn (1948 *et seq.*).

The main control effort against the Desert Locust (*Schistocerca gregaria* Forsk.) is made hundreds of miles outside the crop areas threatened, on locust populations ranging in density from several thousands to less than one per square metre, and the number of locusts killed is accordingly a more appropriate measure of achievement than is extent of infested area cleared. Furthermore, the quantitative evidence now beginning to become available (Rainey, 1956) indicates that Desert Locust numbers are at times still large compared with the maximum control effort so far brought to bear on them. The most appropriate criterion for comparative assessment is therefore considered to be locusts killed per unit of insecticide. This criterion leads in turn to estimates of overall expenditure per unit kill; and, while considerations of cost may appear to the pure scientist as perhaps a little sordid, cost is in fact the best index we have of the not inconsiderable demands made by locust control on the often meagre resources of the communities concerned. Furthermore, such a criterion makes possible comparisons not only between different control methods in the field but also between field and laboratory results, showing the extent to which the potentialities of particular materials and methods are in fact realised under field conditions.

Some of the problems encountered in such assessments and in their interpretation are indicated in the following examples. The simplest type of assessment concerned can be illustrated by a small-scale baiting test carried out on part of a large hopper band, mainly in the third instar, near Lorugumu in Kenya on 26 May 1954, with the help of Messrs. G. Popov and A. J. Wood. At 08.00 hrs., 50 grams of a specially prepared bran bait, containing 4 per cent of a 2.6 per cent gamma-BHC dust, was moistened slightly and applied, at roughly 1 gram per yard, in about half a dozen parallel strips across a narrow stream of hoppers marching at 0.2 m.p.h. along a bare, dry, sandy flood-channel. Two minutes later the stream of hoppers was re-established, and a stationary group had formed along the first bait-line; and by 08.37 hrs. the bait had been completely consumed. Dying hoppers were seen from 08.55 hrs. until noon, and a total of 1.77 kilograms of dead, comprising 1 first-, 1 second-, 6952 third-, and 13 fourth-instar hoppers, were found be-

tween 27 and 400 yards of the bait site. This corresponds to a kill of 134 thousand, or 34 kilograms of hoppers, per gram of gamma-BHC, the highest bait kill we have so far recorded.

The assessment of control operations against adult swarms is complicated by the mobility of the target swarms, which have often travelled more than 50 miles a day, and by the scale of such operations, which necessarily involves problems of sampling (instead of dealing with the complete kill). The complication of mobility can sometimes be circumvented, as in operations against settled swarms in which most of the kill remains on the site sprayed. A convenient procedure in such circumstances is the assessment of a single spray-run across the target area by a series of equidistant traverses at right angles to the spray-line, extending as far as dead are to be found in either direction. Each traverse provides an estimate of kill per yard of spray-line; differences between traverses provide estimates of sampling error; and from the emission-rate and ground-speed of the aircraft the kill per unit of insecticide can be computed. Such an assessment, carried out near Nabadid in Somaliland on 8 September 1955, gave a kill on the roosting site of  $340 \pm 26$  locusts per gram of gamma-BHC (McDonald, 1955b), one of the highest so far recorded in aircraft spraying of completely settled swarms. Where the kill is nearly complete, such an assessment also provides data on the area density and hence on the total number of locusts in the swarm—found to amount to 100-200 million per square mile in a number of such assessments in eastern Africa in 1953-55. Such data are fundamental for any effective consideration of Desert Locust numbers, either in relation to plague dynamics or to control strategy, and have so far been obtainable in no other way.

For assessing operations against flying swarms, the simplest situation is that of spraying a swarm shortly before it settles in the evening, and assessing the resulting overnight kill on the roosting site by counts at regularly spaced sampling points. Such an assessment, already recorded (Rainey & Sayer, 1953), demonstrated the highest kill so far attained against flying swarms, i.e.  $1090 \pm 170$  locusts per gram of gamma-BHC, some 1.5 tons of locusts per gallon sprayed.

In other cases the assessment of operations against flying swarms requires sampling traverses across the route taken by the swarm, to determine the number of locusts falling out per yard of swarm-track. From the corresponding ground-speed of the swarm, given by successive aircraft fixes of its position, the rate of fall-out can be found, in locusts per hour; similar figures are obtainable from a roosting site assessment, knowing the number of hours spent by the swarm on the site; and plotting rate of fall-out against time gives the total kill, by graphical integration. In this way, striking differences in speed of action have been found between insecticides, with half the kill due to DNC sometimes falling out within an hour of spraying (Morris, 1955a), while after roughly comparable operations with BHC, in southern Kenya in January 1954, the rate of fall-out after 24 hours was not much less than that at 4 hours.

A still more protracted kill is illustrated by the assessment of a 3-mile spray-run, to which a 3.6 per cent dieldrin emulsion was applied on 21 January 1955 at 15 gallons per mile, within a widespread infestation of fifth-instar hoppers and fledglings near Wajir, Kenya, which on this occasion continued to cross the spray-line for more than a week and thus provided exceptionally favourable circumstances for the operation of a persistent stomach insecticide. The assessment, in which I was particularly indebted for the assistance of Mr. J. Roffey and Dr. W. J. Stower, was made by periodic counts of dead and dying locusts in regularly-spaced sampling areas along traverses at right angles to the spray-line. The locusts counted were marked and replaced, and counts of the marked and unmarked locusts found on each occasion thus provided estimates not only of the number dead since the last count but also of the corresponding rate of disappearance of corpses by the action of predators and scavengers, found to amount to 40 per cent per day during the earlier part of the assessment. The total kill thus found was 8.4 million hoppers and 5.6 million fledglings, over half of them dying more than two days after spraying, and found over an area extending for 1200 yards from the spray-line. This total kill corresponds to 2600 dead per linear yard of spray-run; and, as the original average density of the locusts within the target area was estimated, visually and photographically, at about 5 per square yard, the effective swathe width cleared was some 500 yards. From the drop-spectrum given by the spray-gear used (determined for us by the Colonial Insecticide Research Unit at Arusha, Tanganyika), and the emission rate, flying height, speed and wind recorded during the

trial, the resulting distribution of deposit density of dieldrin was estimated; and, from laboratory data (MacCuaig, 1956) on the contact toxicity of this dieldrin-xylene emulsion to fifth-instar hoppers (incidentally found to be about one quarter of the corresponding contact toxicity of dieldrin in an oil solution), it was estimated that contact kills of more than 50 per cent could only have been expected within a 6-30 yard swathe. Contact action is accordingly unlikely to have accounted for more than a small proportion of the total kill recorded. Laboratory data on the stomach toxicity of dieldrin to fifth-instar hoppers (Pearson, 1957) have given a median lethal dosage of about 5 micrograms per gram, which would correspond to about 0.5 microgram per square centimetre if applied evenly to the 10 square centimetres of leaf indicated, by comparable field tests on caged hoppers at the time, as a roughly representative figure for the daily food consumption of a fifth-instar hopper under these conditions (Ellis & Ashall, 1956).

Table I summarises the results of all available assessments of baiting and aircraft spraying operations which have provided quantitative estimates of the complete kill attributable to a known quantity of insecticide. The bait assessments relate to small-scale experimental trials in Ethiopia (by Ashall) and Kenya during 1954-55. The airspray data include assessments representative of two seasons' large-scale operations in East Africa (Rainey, 1954, 1956; McDonald, 1955a) together with results obtained in Kenya in 1945 (Gunn, Graham *et al.*, 1948), Tanganyika in 1947 (Gunn, Lea *et al.*, 1948), the Sudan (Morris, 1955a, 1955b), and Somaliland (McDonald, 1955b). In addition to these full assessments, less complete supplementary quantitative data were secured on more than fifty further occasions. The control development work illustrated by these various assessments has posed a series of clearly-formulated questions on locust behaviour, which have incidentally provided effective stimuli to the research work already in progress in this field also.

TABLE I. Summary of Field Assessments of Poison-Baiting and Aircraft Spraying.

	Number of complete assessments	Locusts killed per gram of active ingredient	
		Number	Weight (gms.)
BAITING <sup>b</sup>			
(0.09 to 0.13% gamma-BHC in bran)			
1st instar hopper bands	2	242,000 to 348,000	7000 to 16,000
3rd instar hopper bands	11	100 <sup>a</sup> to 146,000	25 <sup>a</sup> to 34,000
4th instar hopper bands	2	16,200 to 21,900	8000 to 11,000
5th instar hopper bands	10	3 <sup>a</sup> to 22,200	3 <sup>a</sup> to 17,000
SPRAYING			
Extensive infestation 5ths. and fledglings (3.6% dieldrin emulsion)			
	1	1910	2300
Adult swarms, sprayed completely settled, with:-			
20% DNC in oil	3	5.4 <sup>c</sup> to 240	11 to 480
11% gamma-BHC in oil <sup>e</sup>	7	13 <sup>a</sup> to 340	26 <sup>a</sup> to 680
Adult swarms, partly or largely in flight, with:-			
20% DNC in oil	5	22 <sup>d</sup> to 410 <sup>d</sup>	55 to 820
11% gamma-BHC in oil	5	18 <sup>e</sup> to 1090	44 to 2200

<sup>a</sup> Ranges quoted omit two of the baiting trials and one airspray trial, in which no dead were found at all.

<sup>b</sup> Mainly unpublished data of C. Ashall.

<sup>c</sup> Data of Gunn, Lea *et al.* (1948) for *Nomadacris*.

<sup>d</sup> Data of H. J. Morris (1955a, 1955b).

<sup>e</sup> Unpublished data of D. J. McDonald (1955a, 1955b).

Perhaps the most striking feature of Table I is the range of variation of the results. With aircraft spraying, the one recorded complete failure can be reasonably attributed to the negligible deposit density resulting from cross-wind spraying against a settled Somaliland swarm in a 20-knot wind, but the remaining assessments give kills still varying by a factor of 200; and there is good evidence, outlined elsewhere (Rainey, 1956), that the most important variable concerned is the spacing of the target locusts, i.e., the area density of settled swarms, and the volume density of flying targets—in fact the behaviour of the



locusts, the effect of which on the results obtained far outweighs for example any difference between the two insecticides concerned.

The baiting results vary still more, by a factor of over 10,000, even apart from the two complete failures recorded, and after allowing for differences in hopper size. The variation found within instars is much greater than systematic differences between instars; thus half of the assessed kills of third-instar hoppers were less than one-fifth of the best kill recorded against this instar; and only half of the assessments on hoppers of the fifth instar gave kills of more than one-tenth of the best against this stage. While occasional baiting failures have been reported from time to time during control operations (particularly against the older hoppers), both the extent of such failures in practice, and their mechanism, are as yet unknown, though there are indications that behaviour (perhaps meal size?) may again provide the most important variables involved.

In every instar, however, bait kills of over 10 kilograms of hoppers per gram of gamma-BHC, five times higher than the best of the spray kills, have been recorded. There is evidence that this is primarily attributable to a more efficient application of the insecticide in bait, rather than to any inherently higher toxicity by stomach action than by contact. Thus laboratory data on the toxicity of BHC as a stomach poison (Burnand, 1953) indicate a median lethal dosage of about 2.5 micrograms for third-instar hoppers, which suggests, for the kill of 34 kilograms of hoppers per gram of gamma-BHC attained against this stage, the somewhat startling field efficiency of over 50 per cent of that corresponding to the administration of the median lethal dosage under laboratory conditions. An analogous comparison of the best spray kill (2.2 kilograms per gram), with the corresponding laboratory value of 8 micrograms per gram for the contact toxicity of gamma-BHC to adult Desert Locusts (MacCuaig, 1957), gives a field efficiency of only 4 per cent; and the best DNC spray kill [0.8 kilograms per gram (Morris, 1955b)] similarly corresponds, with a laboratory-determined  $LD_{50}$  of 12 micrograms per gram (MacCuaig, 1956a, to a field efficiency of 2 per cent.

In order to obtain from such data, on kill per unit of insecticide, estimates of the corresponding cost per unit kill, it is necessary to have figures for the comprehensive costs per unit of insecticide applied. Such figures are readily available from the total costs and total insecticide applied in representative large-scale campaigns. Typical comparable figures under our conditions have been 1.6 shillings per gram of gamma-BHC applied in bait, and 0.06 shillings per gram of gamma-BHC sprayed by aircraft. Table II shows the range of costs per unit kill given by these figures and the assessment data already presented; the extent to which the high efficiency of application reflected by the better bait kills is offset by the high corresponding costs of application (of which nearly half was for transport of bran) is obvious.

In order to examine more closely the comparative economics of two such methods of control, applied against different stages of the life-cycle, it is necessary to decide how many locusts at the later stage are to be regarded as resulting from each locust surviving at the earlier stage. The result of such a comparison will depend not only on the mortality and reproductive rates concerned between these stages, but also on which method is regarded as having been applied first; the comparative economics of operations against hoppers and adults will usually be considerably different if the hoppers are regarded as of the same generation as the adults or as the progeny of the latter. There is no simple single answer to this problem, and it is suggested that the most useful approach may be to combine the results of making such a comparison in three different ways—first in terms of locust numbers killed, considering each of the two methods in turn as having been applied first; and finally comparing kills by weight, as a third comparison.

As an example, the data available on the comparative economics of a large-scale baiting campaign in a major breeding area, such as that in Ethiopia and the Somalilands in late 1953, and of aircraft spraying operations on a considerable scale, such as those in Kenya and Tanganyika in 1954 and 1955, may be examined in this way. Costs of baiting first-instar hoppers and of aircraft spraying against their parents can first be compared, on the probably not unreasonable assumptions that each female lays a single pod of 80 eggs, that the sex ratio of the parents is unity, and that embryonic mortality may be disregarded by comparison with other uncertainties. Each adult may thus be regarded as equivalent for this purpose to 40 first-instar hoppers in the next generation; the two bait assessments available give



TABLE II. Field Data on Comparative Costs of Poison-Baiting and Aircraft Spraying.

	Number of complete assessments	Range of comprehensive costs* of kill	
		Per million locusts	Per ton of locusts
BAITING			
(0.09 to 0.13% gamma-BHC in bran)			
1st instar hopper bands	2	£0.22 to £0.33	£5.0 to £11
3rd instar hopper bands	11	£0.55 to £800†	£2.3 to £3100
4th instar hopper bands	2	£3.6 to £4.8	£7.1 to £9.6
5th instar hopper bands	10	£3.5 to £26,000†	£4.7 to £26,000
SPRAYING			
Extensive infestation 5ths. and fledglings (3.6% dieldrin emulsion)			
	1	£6.4	£5.3
Adult swarms, sprayed completely settled, with:-			
20% DNC in oil	3	£9.1 to £400	£4.5 to £200
11% gamma-BHC in oil	7	£8.7 to £230†	£4.3 to £114
Adult swarms, partly or largely in flight, with:-			
20% DNC in oil	5	£5.3 to £125	£2.6 to £50
11% gamma-BHC in oil	5	£2.7 to £161	£1.4 to £95

\*From range of rates of kill, given by field assessments, and actual overall costs of representative large-scale campaigns in eastern Africa in 1953-'54 (£70 per ton of bait applied and £2 per gallon sprayed; Rainey, 1954).

†Apart from two of the baiting trials and one of the airspray trials, in which no dead were found at all.

costs of £9 and £13 for killing 40 million hoppers. These figures are to be compared with the costs of £2.7 to £38 per million adults given by the nine relevant airspray assessments. The costs of the two methods, in terms of this comparison, are of similar order; but the duration of the first hopper instar (and hence of the opportunity for such bait kills) is only a few days, while several months are spent in the adult stage.

Comparing, next, baiting against the older hoppers with spraying against the following adults of the same generation, the suggested assumption is to disregard mortality over this period, and to compare the costs of killing equal numbers of the older hoppers (£3.6 to £4.8 per million in the fourth instar; £3.5 to £26,000 per million in the fifth instar, together with one complete failure), and of adults (£2.7 to £38 per million, as before). Compared in these terms, the advantage is clearly with spraying; six of the ten bait assessments on fifth-instar hoppers in fact gave costs substantially higher than the poorest of the corresponding airspray kills.

Finally, direct comparison may be made of costs per unit weight of kill at all stages. While at first sight perhaps a somewhat artificial conception, corresponding to a continuous and exact balance between rates of growth and reproduction on the one hand and of mortality on the other, this is after all the datum line about which all natural populations must ultimately fluctuate. On this criterion, the best bait kill so far recorded corresponds to costs of £2.3 per ton (of third-instar hoppers), with better values (£1.4 and £1.5 per ton of adults) given by two airspray assessments; and the costs corresponding to the poorest of the airspray kills (£19 per ton) were exceeded in two of the ten bait assessments on 3rd-instar hoppers and in seven of the ten 5th instar assessments.

It may therefore be concluded that, in extensive aircraft spraying operations against swarms in East Africa during 1954 and 1955, kills are likely to have varied between limits corresponding to costs equivalent (in terms of small-scale assessments) at worst to some of the poorer (though not the poorest) of the bait kills, and at best fully comparable with those for the best experimental bait kill yet recorded. Furthermore, while there is good evidence for considering the airspray assessment data utilised as adequately representative of the full range of conditions encountered during these actual large-scale campaigns, the small-scale bait assessments differed from routine control operations in that, in all save 3 cases, the whole of the bait applied was consumed by the hoppers, while in practice, rates of application of bait are always heavier than this, by a factor which may be anything between 2 and 10 or more.

It must be emphasised that the conclusions from such a comparison are directly applicable only to the conditions encountered and to the materials and methods used. Uncritical enthusiasts sometimes claim that "Aircraft are the answer" without saying "To which question?", and without fully realising that aircraft, like any other control equipment, demand competent and intelligent operation to give satisfactory results. Far more often, one hears that "Aircraft are so expensive", without consideration of the costs of any alternative methods of doing the same job, or whether such alternative methods even exist at all.

In this particular case the two methods compared are in fact complementary, being applicable at different stages of the life cycle (nymphal and adult), so that any organisation pinning its faith exclusively to one or the other must necessarily be less economic than an organisation using a combination of the two could be.

In conclusion, I would like to revert briefly to the interesting difference of emphasis, particularly in research policy, between grasshopper work on this side of the Atlantic and in our Old World locust organisations, which was illustrated at the Locust & Grasshopper symposium on 20 August. To my mind the essential point of difference is not the somewhat unreal issue of chemical versus ecological control, but rather that in North America the main emphasis of research appears so far to have been given to the immediate job of killing the grasshoppers, while we on the other hand feel that our most important job is that of finding out what goes on with our locusts, as an essential basis for any attempt to deal with them at all.

The object of the kind of work discussed in this paper is to find out how to deal with our locusts as economically as possible, or how to make the best use of the facilities and resources available; in other words, how to give the subscribing communities the best value for their money. And, for that purpose, the first call on the resources available, at least in dealing with the locusts of the Old World, must certainly be to find out what goes on; for against such enemies tactical efficiency is useless without sound strategy.

The work outlined in this paper has been made possible by a degree of co-operation of colleagues in a dozen countries—in laboratories, both government and industrial, as well as in the field—which it is a privilege to be able to acknowledge.

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# The Establishment of Pesticide Tolerances in the United States

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## ABSTRACT

*In the United States, a new law provides a practical method of establishing safe tolerances for residues of insecticides that may remain on food crops. The person who requests a tolerance must supply the Government with information about the pharmacological action of the residues that may remain on treated crops; he must have an analytical method adequate to determine residues.*

*Produce may not be shipped from one state to another within the United States nor from other countries into the United States if it bears insecticide residues in excess of the safe tolerance level. A few insecticides have been exempted from the requirement of a tolerance.*

It is a pleasure and an honor to be present at your meeting and participate in this symposium. I am glad to be able to speak on the Pesticide Chemicals Amendment to the Federal Food, Drug, and Cosmetic Act. This is a law administered by the United States Food and Drug Administration which I represent. The Amendment provides a practical method of establishing safe tolerances for residues of insecticides and other pesticide chemicals in foods. For convenience, I will refer to it as the "new law".

The new law was enacted in 1954. It became fully effective in July, 1956. It provides that a food within its jurisdiction shall not be marketed if it bears a residue of a pesticide chemical unless: 1. The chemical generally is recognized by experts as safe, or 2. Upon consideration of an adequate amount of scientific evidence, the government has established a safe tolerance for residues of the chemical or has exempted it from the requirement of a tolerance, and, 3. If a tolerance has been established, the residues remaining on the food are within the safe tolerance level.

Thus, there are several classes of pesticide chemicals:

First, some chemicals are recognized by qualified experts as being safe as employed today in agriculture. They require no tolerance even though they may leave slight residues on food. Sulfur and lime sulfur are examples.

Second, there are chemicals which leave no residue when applied to crops. Some of the dormant sprays fall in this class.

Third, other chemicals which are poisonous do not constitute any foreseeable hazard to the public health as used in agriculture today. Thus, they may be exempted from the requirement of establishment of a tolerance. Over 20 such pesticide chemicals have been exempted in the United States when they are applied to growing crops. They include petroleum oils; a number of the common copper compounds, such as Bordeaux mixture; pyrethrins; four common pyrethrin synergists; and related substances. These materials are not exempted when applied after harvest.

Fourth, there are poisonous insecticides which may constitute a hazard to the public health unless they are used in such manner that the quantity of the residue on food is controlled. These materials require the establishment of a safe tolerance, and protection of the public health requires some government checking to determine that the tolerances are not exceeded. Most of the insecticides in use today fall into this class. A number of tolerances for such compounds already have been established, and others are being established.

The new law requires that the person who requests establishment of a safe tolerance for insecticides in this fourth group submit data to the government to support the application. Often, the manufacturer requests the tolerance, although others may do so. The information required to support a request is:

1. *The name, chemical identity, and composition of the pesticide chemical.*
2. *The amount, frequency, and time of application of the pesticide chemical.*
3. *Full reports of investigations made with respect to the safety of the pesticide chemical.*

The toxicity of a new insecticide must be determined by animal experimentation. Acute and subacute toxicity studies are valuable, but before a formal tolerance is established, they must be supplemented by chronic oral-toxicity studies.

Chronic-toxicity studies generally are regarded as lifetime or two-year feeding studies in the rat, and a one-year, or longer, feeding study in the dog or monkey (or perhaps some other test animal). Such long-term tests are required because the possible effects of the lifetime ingestion of insecticide residues by man cannot be predicted reliably with tests less stringent.

The results of toxicity studies on animals, together with supplemental data such as the effect of the poison on humans where human exposure has occurred, are evaluated to determine whether there is a level of feeding of the insecticide which should cause no harm to man if continued throughout his lifetime.

4. *The results of tests on the amount of residue remaining when the chemical is applied according to proposed directions. This should include description of the analytical method used.*

It would be futile and unwise to establish a tolerance for an insecticide if its effective use would leave residues on food in excess of the tolerance.

Additionally, the public health can be safeguarded only if analytical methods are available for making trustworthy analyses for the minute residues that remain. The new law places upon the manufacturer the responsibility of developing an analytical method which is adequate to determine residues of the insecticide.

5. *Practicable methods for removing residues which exceed any proposed tolerance.*

The petition also should state the tolerance or exemption requested and reasonable grounds in support of the request.

When the petition is filed, it is the government's responsibility, within a definite time limit, to establish a tolerance for residues of the insecticide, or an exemption. The tolerance may be zero if the facts do not justify a higher value.

From results obtained in feeding experimental animals and from effects on man where human exposure has occurred, experts can predict the quantity of a poison that may be consumed over a long period without hazard. It is reasonable and proper in making the prediction to assume that man is ten times more susceptible to injury from the substance than other species of warm-blooded animals, and that the most sensitive men are ten times more susceptible to injury than the average man.

When the pharmacologists have evaluated the chronic-toxicity data and have applied the necessary safety factor, and estimated what level of residue will be safe for man, and when the chemists have studied the analytical methods for detecting residues, have determined that they are adequate, and have studied the residue data, then the government is ready to establish safe tolerances for residues which may remain. The following principles are observed at this time:

1. If the quantity of the insecticide that may be contributed to the diet from all sources, including that derived from residues on food, exceeds the quantity estimated to be safe, the tolerance is set at the point of estimated safety.

2. If the total quantity of the insecticide that may be ingested from all sources does not exceed the quantity estimated to be safe, the tolerance is based on the quantity of the residue which is needed to protect the crop.

3. In case two or more insecticides have a related or cumulative effect on man or test animals, the tolerances for these materials must be set so that the total allowable residues for all such related compounds will not exceed a safe figure. For example, it is proper to set tolerances for residues of two or more arsenicals in terms of the total amount of arsenic which may remain and it is proper to limit the combined residues of two or more chlorinated hydrocarbons which may remain on a crop.

The new law provides also for the establishment of temporary tolerances to permit the marketing of foods produced on large scale experimental plots.

The tolerances that are in effect in the United States apply not only to food produced there but also to food shipped from another country to the United States. Olives from the Mediterranean region, fruit from South America, hay and wheat from Canada, and any



other food commodity being shipped into the United States must meet the tolerance requirements of that country.

Thus, it is important for those who expect to market their crops in the United States to know what tolerances will apply. Let me illustrate the point:

Parathion has been considered for use in the Mediterranean region to control the olive fly or *Dacus* fly. When used so that it gives adequate control, it leaves residues approaching 3 parts per million. The presence of 3 ppm of parathion in olives or in olive oil would not constitute a hazard to man provided the chemical were not present in other foods. It is entirely possible that the Mediterranean countries will decide to recommend parathion for control of this pest of olives. In the United States, however, we permit parathion to be present on 59 fruits and vegetables. Because these represent a very significant portion of the diet, we permit only 1 ppm of parathion on these crops. This tolerance applies to olives. Olives may not be shipped into the United States if they contain more than 1 ppm of parathion. Thus if the Mediterranean countries decide to use parathion so that it leaves higher residues, they should not ship the olives containing these high residues to the United States, nor should they ship oil prepared from such olives because all indications are that the parathion will be concentrated in the oil and will be higher there than in the unpressed fruit.

Material quantities of apples, pears, and other fruits are shipped to the United States from South American countries. These shipments must meet the tolerance requirements. During the past winter and spring, we investigated some shipments of these fruits and found a few that bore pesticide residues exceeding the tolerances. These lots were not allowed to enter the country.

Considerable quantities of hay are shipped from Canada to the United States. We have established tolerances for pesticides on forage crops. We are particularly careful to guard against residue levels of poisons on hay which will contribute significant residues in milk if the hay is fed to dairy animals. There are some chemicals that must not be present on forage going to dairies. DDT is one of these. The feeding of any level of DDT to cows results in contamination of milk. It is evident that if the Canadian farmer has a residue of DDT on his forage crops, they may not legally be shipped into the United States.

And there are a number of other chlorinated hydrocarbons that should not be on hay when it is shipped.

Information about the tolerance levels permitted on crops may be secured from the United States Food and Drug Administration, Washington 25, D.C.

## DISCUSSION

H. M. ARMITAGE. What is the purpose and justification for establishing tolerances at levels often far below those known to be absolutely safe to man and animals, when such action results in a multiplicity of situations which, from the practical standpoint, can neither be observed nor enforced?

W. B. RANKIN. While we are satisfied that the tolerances being established are safe, we cannot overlook the fact that the proof of safety ordinarily depends upon the results of studies on experimental animals, not on man. Frequently, different species of animals react in different ways or in different degrees to chemicals. Thus there always is some element of uncertainty in estimating on the basis of animal studies a feeding level of a poison that will be safe for man. We think it is in the interest of public health to base the tolerance on the quantity of residues which is needed to permit useful employment of the pesticide in agriculture, where this quantity is below the level estimated to be safe. We are not establishing tolerances that result in a multiplicity of situations which can neither be observed nor enforced. Before we establish a tolerance we assure ourselves that it can be met when directions proposed in the petition for use of the pesticide are followed.

H. M. ARMITAGE. In the enforcement of established tolerances is some leeway allowed, viz: if the tolerance is 1 ppm, would 1.001 be allowed?

W. B. RANKIN. Before taking legal action against a shipment because of excessive residues we will assure ourselves that there actually is a residue in excess of the allowable tolerance. For example on many crops there is an analytical error in determining DDT of about 10%. Thus if an analytical result shows exactly 7 ppm of DDT there may actually

be from 6.3 ppm to 7.7 ppm of the chemical present. We would not take action unless the analysts were able to testify that there definitely is a residue exceeding the tolerance.

Once the analytical results show that the tolerance has been exceeded, after due allowance is made for analytical error, we believe that the law permits legal action without provision for any tolerance on top of a tolerance.

H. M. ARMITAGE. If 7 ppm is established as the tolerance for a given insecticide on a given agricultural commodity why is not that tolerance acceptable on all agricultural commodities?

W. B. RANKIN. The tolerance may not be acceptable on all commodities because this could permit more of the chemical in the diet than is judged safe. An illustration given in my paper is in point: We have no doubt that a residue of 3 ppm of parathion on olives is safe provided the chemical appears only on olives, but when parathion is tolerated on 59 fruits and vegetables, as is the case in the U.S.A., we believe that a safe tolerance level is 1 ppm. The lower level is needed to restrict the total intake of parathion by man.

J. A. FREEMAN. Do the insecticide tolerances apply to export commodities, especially grain and flour?

W. B. RANKIN. The United States law permits exportation of a commodity which would not be legal within the United States provided it:

- (1) Accords to the specifications of the foreign purchaser,
- (2) is not in conflict with the laws of the country to which it is intended for export, and
- (3) is labeled on the outside of the shipping package to show that it is intended for export.

J. A. FREEMAN. To what extent are imports sampled chemically to see whether they comply with tolerances?

W. B. RANKIN. We spot-check imported foods to determine whether they meet the requirements of the United States laws. If this spot-check indicates that a certain commodity may be in violation of the law, we increase the attention given to that commodity.

J. A. FREEMAN. Explain the difference in treatment of stored grain and growing crops with regard to setting of tolerances, especially for pyrethrins and piperonyl butoxide.

W. B. RANKIN. Pyrethrins and piperonyl butoxide have been exempted from the requirement of a tolerance when they are applied to growing crops because the weathering to which they are exposed rapidly removes or detoxifies remaining residues. They are not exempted when applied at time of or after harvest because the safeguards afforded by weathering may not be present. We deemed it necessary in the interest of public health to establish safe tolerance levels that must not be exceeded on the grains.

# Influence of Legal Insecticide Tolerances on Industry

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## ABSTRACT

*The first legal residue tolerance for any insecticide was established in Manchester, England, in 1900. Laws providing for legal insecticide tolerances in the United States are the Food, Drug and Cosmetic Act of 1938, and the Miller Pesticide Residue Amendment to the Food, Drug and Cosmetic Act, passed in 1954. Establishment of tolerances on a scientific basis allows growers to use insecticides effectively and, at the same time, assures consumers that foods protected by the use of insecticides are safe. The research and testing of materials to assure both effectiveness and safety are expensive. A number of companies estimate that the total cost of producing a new insecticide is about \$1,200,000. Nearly 60 percent of this expense is for field testing, developing analytical methods, and determining toxicity status for the chemical.*

Before commenting on the influence of legal tolerances on Industry of the Miller Pesticide Residue Amendment to the Food, Drug, and Cosmetic Act (Public Law 518, 83rd U.S. Congress, 1954), some background information of the so-called residue problem, I believe, is in order, as the legislation involves not only technical problems of entomology, toxicology, pathology, and similar sciences, but equally important, a public understanding of the legislative intent.

According to the best information we have, the first residue tolerance was established for arsenic. This took place in Manchester, England in 1900 following a series of poisonings attributed to arsenic-contaminated beer. After a very careful study the British Government established a tolerance of .01 grams of arsenic per pound of solid food. This equals 1.4 parts per million. Until 1926 no serious effort was apparently made by the British Government to enforce and apply the tolerance, but about that time it was applied to apples shipped from the United States. It was reported that poisonings in England came from the American fruit product. Like many other reports the facts were somewhat hazy, and the poisonings were considered unlikely by the shippers. However, since that time the tolerances established have been carefully observed by American exporters to the British Empire.

The residue problem is not new in the United States, and the Food, Drug, and Cosmetic Act of 1938 carried a provision providing for the establishment of legal tolerances on pesticide chemicals required in the production of food.

Several informal tolerances on materials in common use during the 30's were established and recognized by the Government agencies, agricultural interests, and the Industry.

In 1947 the Federal Insecticide, Fungicide and Rodenticide Act, replacing the original Insecticide Act of 1910, was passed by the Congress of the United States. This Act, together with the Miller Pesticide Residue Amendment to the Food, Drug, and Cosmetic Act, provides for registration before sale of agricultural pesticides, and adequate pretesting to determine permissible safe pesticide residues, if any.

Compliance with these laws assures consumers that raw agricultural products produced in the United States are safe, and producers are assured that pesticide chemicals are safe when used in accordance with approved label recommendations.

Pesticide chemicals are the most thoroughly tested chemicals marketed today.

I want to point out, however, that the passage of the Miller Pesticide Residue Amendment did not in itself bring about the situation of safe raw agricultural commodities and the safe use of pesticides. Reputable manufacturers of pesticides have for many years conducted toxicological and residue work on their products. Prior to 1954 both the Food and Drug Administration and the U.S. Department of Agriculture had informally reviewed research work and informal tolerances had been observed by the Food and Drug Administration, the Department of Agriculture and the Industry.

The Miller Amendment simply sets forth a new procedure for the establishment of tolerances, and eliminates lengthy public hearings and other procedures which were found impractical in operation under the original law.

Why all the agitation about residue hazards? Unfortunately, there has been a great amount of unfavorable publicity about the hazards of pesticide residues apparently sponsored by food faddists, so-called authorities, and in some cases, by selfish commercial interests. This unfavorable publicity has created, in the minds of some of the public, a fear of the use of these chemicals.

We have never been able to substantiate any case of illness caused by a pesticide residue when the products of the Industry have been used according to directions and in accordance with good agricultural practices. A study of the vital statistics shows that these products have an excellent safety record.

I want to quote from two statements: one made by the former Surgeon General of the United States, Dr. Leonard A. Scheele, and one by the Assistant Surgeon General, Dr. David E. Price.

Dr. Scheele said:

"Using today's armaments of insecticides, sanitation facilities and other control measures, it is possible to prevent the spread of diseases carried by these insects.

"Some 5 million lives have been saved and 100 million illnesses prevented since 1942 in this country by the use of insecticides.

"In addition to the high cost and burden of human sickness transmitted by disease-carrying insects, other insect enemies are causing an annual \$4 billion loss. They damage agriculture crops, carry disease to farm animals, and they destroy more trees each year than are destroyed by forest fires."

Dr. Price said:

"Insecticides, particularly DDT, have been alleged to be responsible for a variety of gastrointestinal complaints, a wide range of psychoneurotic disturbances, as well as for an increase in poliomyelitis, cancer, and other diseases.

"The vast majority of physicians and other scientists who have studied the problem do not accept these claims. The consensus is that, properly used, insecticides do not cause any diseases or increased susceptibility to disease in either man or animals. This opinion is based on the results of extensive research."

In spite of this safety record, however, and the care used by the Department of Agriculture and the Food and Drug Administration during the past few years, the Industry, Government agencies, and the National Safety Council, are conducting a vigorous program on the safe use and handling of pesticide chemicals. We are striving to make our safety record, both from the viewpoint of the grower who handles the materials, and the consumer of the food product, a perfect one.

Another important fact—too few people realize that pesticides are necessary in the production of our food crops. A recent survey shows that 80 major food crops in the United States can not be produced without the use of some pesticide chemical. This list of 80 crops does not include any of the crops in surplus in the United States at the present time. These crops must be protected not only during production but also in transit and storage. We could not have the full nutritional supply of food stuffs available if the use of pesticides was unduly restricted.

Pesticides are more important to the agricultural interests of the United States and the economy of the country than even to the pesticide industry. The sales volume of pesticides in the United States at the manufacturers' level is estimated at \$200 million, but this small investment protects close to \$30 billion of farm production.

Everyone interested in pest control should keep in mind the following facts as background for any legislative activities: 1. Pesticide chemicals are necessary to produce food. 2. The wide varieties and low costs of our food products would not be possible without pesticide chemicals. 3. When used as directed, in accordance with good agricultural practices, pesticide chemicals are safe. 4. Pesticides used in the production of raw agricultural commodities are thoroughly investigated by Industry, and their research is reviewed and approved by the Department of Agriculture and the Food and Drug Administration before registration for sale.

Now let me comment specifically on the impact on Industry of the Miller Pesticide Residue Amendment to the Food, Drug, and Cosmetic Act. We believe that the law is basically sound, and we recently advised the author of the Amendment, Dr. A. L. Miller, Congressman from Nebraska, that at the present time we have no amendments to suggest to it. It is the consensus of the Industry that the law is basically sound, and that practical administrative procedures will be adopted as we gain experience in its operations. We hope



to make this a model of Government-Industry cooperation in the protection of the public and increased agricultural production and prosperity.

No law, however, is any more effective than its administration and the Government agencies are cooperating with Industry to make the law work effectively in the interests of the public and in the interests of the farmers who use pesticide chemicals.

Much progress has been made in the administration of the law, and further progress is anticipated before the beginning of the next crop season. There are, however, a great many legal, technical, and practical problems still to be solved.

Recently, we conducted a survey among our members asking for comments, criticisms and suggestions on any problems arising due to the legislation and regulations thereunder. The purpose of this survey was to find any flaws which might exist and to clarify them before they could become of major importance. We received approximately ten suggestions which, in due course, will be discussed with the Government agencies responsible for the enforcement of the law. It appears to us that this reflects considerable progress when the highly technical nature of the problem is considered.

I believe that the following quotation, which is typical of replies received to our Industry survey, best expresses the over-all opinion of Industry:

"The above comments are all criticisms of the present system. We do want to add to these our expression of appreciation to both USDA and FDA for the conscientious efforts they have made to be fair and realistic as far as present policy will permit, and always to be willing and helpful. We hope the above comments will be considered and that they will be found useful and acceptable. We have all struggled through the first year of the Miller Bill, making mistakes, feeling our way and I hope learning as we went along. We will be interested in any comments, suggestions, or criticisms the USDA and FDA may have that will help us to improve the program."

In general, the problems which will be reviewed relate to such subjects as the amount of toxicological data necessary, the amount of residue data which must be made available before a tolerance is established, the interpretation of data from one crop or animal to another, and the relative degree of accuracy required in the analytical method submitted at the time of registration. This becomes an important, practical, as well as technical, problem when materials must be used on crops of limited acreage, but where the cost of research does not warrant a company's conducting research for a particular use.

Research cost constitutes a major problem. It is high—almost fabulous. One of the major companies in our Industry estimates that research costs, involved in developing a new pesticide chemical for the commercial market, amount to about \$1,200,000. This is corroborated by the experience of a number of companies in the Industry. I want to quote from a recent talk by Mr. W. W. Allen of The Dow Chemical Company, President of our Association, which indicates the cost of research

"Recently the cost of research to develop a single insecticide was estimated just under One and One-Half Million Dollars. Under some circumstances, the cost might not run that high. A break-down follows:

"To synthesize the compound and run the necessary screening tests . . . . .	\$ 70,000.00
"To study the patent situation and file patent applications . . . . .	8,000.00
"For testing in the field to see how it works under farm conditions, the effect on crops as well as bugs . . . . .	just under 500,000.00
"To set up a pilot plant in which to produce enough for large-scale testing . . . .	50,000.00
"The development of analytical methods . . . . .	130,000.00
"To determine the toxicity status of the material . . . . .	75,000.00
"To determine how to formulate a new chemical . . . . .	100,000.00

"There are additional costs for fees for setting of tolerances, assembly and presentation of data, etc. These items do not include the capital cost for a plant, which ranges from Four to Seven Million Dollars."

At this time it is impossible to determine the long-range effects of the legislation on the Industry. If the technical requirements are too severe, development of new products for new uses will be curtailed due to high research costs. Already some highly promising materials have been withdrawn from further experimental work.

Opinions at the present time vary on the long-range effects.

In conclusion, I want to express a view which is borne not only by the Industry but, I am quite confident, also by the Government agencies. The success of the legislation depends primarily upon education. The purposes, objectives, and the operations thereunder must be understood not only by manufacturers and the Government agencies but also by the research workers, and finally by the growers and the public.



# Influences of Legal Insecticide Tolerances on Research

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## ABSTRACT

*The problem of insecticide residues on raw agricultural commodities is handled in different ways depending upon the country involved. Legislation recently enacted in the United States known as the Pesticide Chemicals Amendment to the Food, Drug and Cosmetic Act, has had several noticeable effects on research in the insecticide field. Among others, it has become essential to obtain residue data to support recommendations for the use of pesticides in agriculture, as well as to support tolerance and registration claims for the pesticides themselves. Legal tolerances have expanded the research on analytical methods for residue determination, and increased the need for research data on acute and chronic toxicity levels of pesticidal chemicals to warm-blooded animals. Because of development costs running as high as a million or more dollars for a single product, industry must constantly evaluate its research procedures for product development. Thus far there seems to be no marked adverse effect on new pesticide development because of legal tolerances; however, the future may prove otherwise. Because of research on analytical methods, the investigation of mode of action of insecticides has been stimulated.*

*It is noted that raw products produced abroad and entering the United States must meet the residue tolerances established in that country for any pesticides that may have been used during production.*

One segment of the problem of legal tolerances established for pesticide residues in or on raw agricultural commodities deals with the effect on research. Since, in the United States, we are dealing with legal tolerances recently established, the observations reported in this discussion will be based largely on this experience. Possible effects of an international nature will be brought out.

Barnes (1953) has covered in a comprehensive way several aspects of the use of toxic chemicals in agriculture with particular reference to the toxic hazards of certain pesticides to man. A careful study of toxic chemicals in agriculture, dealing specifically with residues in food, has been made in England (1953). A recent report reviews the effects of chemicals on wild life (1953). Numerous other papers relating to the field have been published, indicating a general interest in the problem in several countries. The Pesticide Chemicals Amendment to the Food, Drug and Cosmetic Act, passed in 1954, has been appraised and effectively summarized by Rankin (1956).

In order to comply with the Pesticide Chemicals Amendment, several changes have taken place in agricultural research dealing with pesticides in the United States. Among the topics for consideration are: (1) Initiation and expansion of pesticide residue research programs in the state, federal, and industrial laboratories. This research is needed to support requests for tolerances needed for registration of pesticides, and to guide farmers in the use of pesticides to avoid excessive residues at harvest, as well as to prevent mis-use. Data on residue levels at harvest are important in requesting tolerances, since the tolerance granted may not necessarily reflect the toxic hazard of the chemical, but in some instances the minimum level of residue needed to control the pest. (2) Legal tolerances have expanded the need for research on methods of analysis for pesticide residues. (3) Legal tolerances have expanded the need for research on acute and chronic toxicity levels of pesticide chemicals to warm-blooded animals. These data are required for the establishment of a tolerance. (4) A greater volume of pesticide chemicals in the environment as a result of increased use provide need for research on the effect of these chemicals on natural balance. (5) Legal tolerances have also forced changes in field experimentation procedures with candidate pesticides. (6) Possible effect of legal tolerances on future development of pesticide chemicals. (7) Miscellaneous effects of legal tolerances on research. Perhaps a discussion of these points may expand their relationship to the general topic.

## REVISION OF PROCEDURE FOR ESTABLISHING LEGAL TOLERANCES IN THE UNITED STATES

In the United States, by the late 1940's it became obvious that the Food and Drug Administration would be called upon to establish tolerances or exemptions as a guide to

safe use of the rapidly developing array of synthetic organic pesticides. The Administration possessed the legal authority to undertake this step, and a series of public hearings during 1950 demonstrated the necessity for revision of the procedure for prompt establishment of tolerances. In 1954, the Pesticide Chemicals Amendment, known also as the Miller Amendment after its sponsor, was passed, and amended the Food, Drug and Cosmetic Act of 1938.

#### NEED FOR RESIDUE DATA

As a need for residue data became apparent, many of the state and federal research programs in the agricultural field were modified to include facilities and personnel for residue investigation. They are still expanding. More and more of the states, along with the federal government and industry, are determining pesticide residues on a part or all of their treated commodities. In order that a chemical used in a pesticide formulation may be registered, an analytical method must be submitted with the petition for registration. Workers soon found that even though a basic method had been developed, often times it had to be adapted, or modified for different commodities. Perhaps oils, pigments, or waxes in different commodities necessitated this additional research on methodology before reliable residue data could be obtained. Today projects of regional interest, involving a number of states in a major producing area, are supported with federal regional research funds plus state funds and federal funds assigned to states for agricultural research. They are tackling research on broad mutual problems in the residue field. Industry is cooperating through technical and financial contributions. The goal is the same for all—to obtain reliable data pertaining to residues at harvest to serve as a basis for establishing a tolerance, or exemption from the necessity for one, for those pesticide chemicals essential to the production of raw agricultural commodities. Further, these data guide recommendations for the use of pesticides by farmers. The financial investment in pesticide residue research is enormous, yet no responsible agency in the United States concerned with pest control in agriculture, be it government or industry, can afford to ignore the responsibility of complying with tolerance regulations. For the foreseeable future, pesticide residue research will be needed to guide our control program as well as those in the developmental stage.

#### RESEARCH WITH CANDIDATE PESTICIDES

Legal tolerances for pesticidal chemicals in the United States have caused renewed consideration of research procedures in the conduct of field evaluation studies for candidate materials. In reviewing practices of the past, it is obvious that many experimental workers took candidate chemicals into field testing without consideration, in all cases, of the disposition of the treated commodity. Many, in fact most, of the field plots are small, and the amount of produce available at harvest is often negligible. With the dilution factor of small plots being mixed into total harvest, the possibility of difficulty from any remaining residues is remote.

In present planning for field research, however, most government and industrial investigators are considering the disposition of treated commodities at harvest as an integral part of planning research with new compounds or formulations. To be on the safe side, it is realized that farmer cooperators, who are invaluable in field experimentation, should not be asked to assume any risk in marketing crops from experimental plots. It is not only desirable that this relationship be continued between the research workers and growers, it is essential in many instances because it is impossible for experiment stations to have and to maintain a variety of crops and locations for pest control purposes. This is particularly true with a diversity of crops, pests that fluctuate seasonally in abundance, and with high value, long-term production as with many of the tree fruits. Often, through the extension workers, research personnel are advised of infestations in various parts of the state where investigators can move in quickly for field plot work.

Current research practices are tending to limit large-scale field testing to chemicals that have a tolerance or exemption established, or for which sufficient evidence is available to obtain a temporary tolerance for specific crops that are to be under experimentation. These compounds which are past the screening stage in industry, yet require more biological evaluation before the industry makes a decision about further developmental work, including long-term toxicological research as a basis for determining chronic toxicity levels, are being reduced to small plot experiments where the harvested commodities can be purchased or otherwise kept out of trade channels. In instances where the plots are on ex-



periment station grounds, there is no problem since usually they are not harvested for market.

Some judgment still must be exercised with regard to the nature of the candidate chemical under experimentation. If evidence from related compounds, its chemical properties, date of application and the like, indicate that there is no reasonable expectation for residues to remain at harvest, treated commodities from experimental plots may be sold. This seems to be a matter of decision for the responsible investigator as well as a policy to be determined by his organization. As with any human endeavor, not everyone follows the same policy where a choice is to be had, but in a general way, the trend is definitely toward keeping commodities from plots treated with experimental materials under strict control until data are available to formulate a different policy.

Companies normally located outside of the United States, but considering market possibilities for their products in this country, likely will expand some of their screening and field evaluation studies in areas that have less rigid legal restrictions on registration of products and, as a result, bring to investigators in the United States information on fewer chemicals than otherwise might be the case. This possibility has been discussed, since it could delay experience on the part of research personnel in the United States with promising candidate compounds and possible areas of usefulness for them. Certainly it would not prevent their ultimate acceptance, and any delay could be more than offset by careful screening of candidate chemicals before they enter our laboratories.

Often it is difficult to know the full potential of a compound without rather extensive field experimentation. If this background can be had before the long-range toxicological work is started, developmental costs may be reduced.

#### EFFECT OF TOLERANCES ON DEVELOPMENT OF NEW PESTICIDES

From the beginning of tolerance legislation, there has been concern that the necessity for tolerances, involving the greater expense in developing a marketable product, would discourage industry in the agricultural chemical field and consequently reduce, or slow down the introduction of new pesticides. Recent experience with a variety of mites and insects that have developed a tolerance or resistance to currently recommended compounds, and the necessity for constantly evaluating new control measures either in advance, or immediately after effectiveness of current recommendations begins to deteriorate, makes this concern of more than passing interest.

It is perhaps too early to pass judgment on this possibility. Up to the present, at least, legal tolerances do not appear to have had a marked detrimental effect on research and development of new pesticides. For the reasons stated, new materials will present a higher cost to the company developing them than might have been true before. It is estimated in the United States that all costs considered in developing a pesticide to the marketable stage may run between \$500,000 and \$1,000,000. Now that a pattern of development is known, firms seeking long-range business in the agricultural pesticide field will consider the demands of tolerance requirements as a part of their developmental program. Acute and chronic toxicity data must be obtained; an acceptable analytical method for residue determination must be developed. These requirements likely will lengthen the development program in industry and may in turn shorten the trial period in the experiment stations before grower acceptance. The increased cost in the long run doubtless will be passed along to the consumer. It may even assist an industry to reach an early decision as to possibilities between several candidate chemicals. It has been said that an industry faces the necessity of getting good return on its investment within a few years after its product is put on the market due to the possibility of resistance developing, competition providing either a cheaper or more effective compound, a major change in the methods of application, and other related factors. The nature of cross-resistance may also have a retarding effect on new compounds, since many of the candidate chemicals are related structurally to the old ones.

One fact is obvious—the pesticide manufacturing and marketing field is different from many other segments of the chemical industry. The market is less predictable in terms of volume and continued use of a given compound. The biological factor of fluctuations within pest populations plays an important role. Many growers still wait for injury to develop before undertaking control. If serious outbreaks appear, the demand for insecticides

can be immediate and extensive. If they do not appear, a heavy inventory of pesticides may build up in the dealer's warehouse. Because of this, and our basic lack of understanding of the nature of resistance, and destruction of natural or biological controls through the use of pesticides in the environment, there is a stimulus to increase research in the fields of insect physiology, toxicology, and ecology. This should provide data upon which more accurate predictions relating to the nature of pest species can be made.

#### EFFECT OF TOXIC CHEMICALS ON WILDLIFE AND NATURAL BALANCE

Among the research problems created by the use of pesticide chemicals in the environment is their possible harmful effect on fish, game, parasites, predators, and other organisms which enter into the so-called natural balance. One might question whether this area of research is affected by legal insecticide tolerances, but through widespread, and ever-increasing use of pesticidal chemicals, their effect will be felt. It is to be hoped that as investigation provides factual information on the effects of chemicals used in the environment for pesticidal purposes, it will be possible through timing, formulation, rate and method of application, selective pesticides and other means available, to minimize the risk to wildlife and other naturally-occurring species that are present within the areas to be treated.

#### EFFECT OF TOLERANCES ON FOOD COMMODITIES FROM OTHER COUNTRIES

Those who have followed recent developments in the United States realize that commodities that come within the interpretation of raw agricultural commodities under the Food, Drug and Cosmetic Act will have to meet residue requirements of the United States even though these commodities are produced in other areas of the world and shipped in. This fact will force more consideration of pesticide residues on commodities produced abroad and shipped to the United States.

#### OTHER CONSIDERATIONS

Legal tolerances have spurred research for methods of analysis and as a result, we now depend on chemical methods, bio-assay methods, and radio-active tracer methods for residue determination. Although the basic method is developed by industry, it is adapted, or improved, by workers in all segments of the field. Research on analytical methods is an expanding field of endeavor.

The study of residues in their own right, has been greatly implemented by the demand for tolerance data. Ordinarily much of this work would have been neglected due to the lack of time and money. Persistence curves and accompanying phenomena, translocation problems, and metabolism of pesticides by plants and animals, are being investigated at a rate greatly in excess of what could be expected without the impetus of residues for tolerances.

Registration for use of a given pesticide may be restricted to the crop, or crops on which reliable residue data are available. Where a crop is of minor importance nationally, but of major concern to local producing areas, state laboratories are filling the gap in providing the residue data to industry for the registration of a product they need and wish to recommend. It appears likely that more of this type of research will be called for in the future.

A problem of magnitude for which no final solution has been devised is how effectively to pool residues research information to make it available to all for whom it is of value. This is under study at present. A national approach will have to be taken, but it offers complicating factors. Local data will always be valuable to immediate areas from which it was obtained. Residues in milk, meat, poultry, and eggs can be transferred from one producing area to another with more reliability than some crop data that are influenced by local climatic conditions and cultural practices.

The establishment of legal tolerances caused food processors in the United States to determine residue levels on their raw agricultural commodities and processed food as a means of assuring themselves that the processed foods will not have pesticide residues which are in excess of the levels established for raw products. This phase of residue research is being assumed by the processing industry. Chemical as well as bio-assay methods are being developed through joint investigation with industry, government, and universities. Methods for reducing residue levels on raw products before processing are being studied.

The protection of processed food supplied from sources of contamination, both residues and pest fragments, is an important contribution to the public health.

Data from chronic toxicity studies required for establishing tolerances or exemptions offset scare stories and unfounded propaganda about the hazard of pesticide residues in the diet. Commodities produced with the use of pest control chemicals for which legal tolerances or exemptions have been established, and used according to approved label directions, offer no health hazard to consumers. Legal tolerances are also reducing farmer experimentation with pesticides because of the risks involved in the complicated pest control programs.

#### SUMMARY

Legal tolerances for pesticide residues have changed the procedures in research in the development of a pesticide chemical for agricultural use in the United States.

Attention is being given in other countries, to residues through the effort of the industries developing them and through their governments. Many countries require registration of agricultural pesticides with a government agency, others have voluntary or no registration. Several countries have legal means for preventing contaminated food, which might include chemical residues, from reaching the market.

Legal tolerances may tend to extend the appraisal period of a new candidate pesticide by requiring acute and chronic toxicity research, the development of an analytical method, and residue research, before industry released these new compounds for experiment station use.

Agricultural pesticides involve a long-range program of research and development. It seems likely that only those companies who incorporate such a program in their operations will prosper in the field.

Legal tolerances have created a widespread program of pesticide residue research in the state and federal experiment stations of the United States, in what appears to be a permanent part of their pesticide research programs.

Legal tolerances have stimulated research on analytical methods which make possible fundamental research on the fate of a chemical after application.

It is felt that in years ahead, an international approach to the residue tolerances may be required because of the implications in world commerce arising from the use of pesticides for food and fiber production.

Residue tolerances in the United States recognize the need for pesticides in production of an adequate, wholesome food supply. They provide proper guidance for the farmer and assurance of safety to the consumer. Proper use of pest control chemicals will contribute ever more to man's well-being as the world population increases. With tolerances or exemptions established for some 95 pesticidal chemicals (Rankin, 1956) future pesticides in the United States will conform to the requirements for legal tolerances or exemptions before they are introduced into widespread agricultural use. The future magnitude of the research will depend on the number of chemicals and uses that are developed.

Legal tolerances have increased research on acute and chronic toxicity of pesticide chemicals to warm-blooded animals and, indirectly, their effects on the natural balance in the environment.

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# Canadian Views and Policies on Insecticide Tolerances

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## ABSTRACT

*The Food and Drug Directorate is the agency responsible for the establishment of tolerances for Agricultural Chemicals in Canada. The particular Section of the Food and Drugs Act under which authority is granted for the setting of tolerances and the legal interpretation applied to it are described. Certain definite requirements for the establishment of tolerances in Canada have been made. These requirements and the administrative procedure for the processing of applications are discussed at some length.*

*Some views on cooperation with other agencies in other countries engaged in similar work are expressed.*

Mr. Chairman, Ladies and Gentlemen: I am very glad to take part in this symposium. During the next few minutes I will endeavour to explain to you some of the views and policies of the Canadian Food and Drug Directorate on the establishment of insecticide tolerances.

First of all I would like to make it clear that the Canadian Food and Drugs Act does not distinguish between an insecticide, fungicide, or other types of pesticides. They are considered as one class of substance under our Act, namely, as poisonous or harmful ingredients which may occur in or on food. Another possible misconception may be that a more or less complete revision of our Act and Regulations has had to be undertaken in order to set tolerances for the new agricultural chemicals. This is not the case. For many years we have had the authority under certain Sections of our Act to set tolerances. As a matter of fact, tolerances for a number of chemicals in or on certain foods have been in our Regulations for a number of years. These are for arsenic, lead, copper, zinc, and fluorine.

For those of you who may not be familiar with the particular Sections of our Act from which our authority to set tolerances is derived, I propose now to review these for you, as well as tell you just what our requirements are for setting tolerances.

## LEGAL ASPECTS OF THE ESTABLISHMENT OF TOLERANCES

Section 4(a) of the Canadian Food and Drugs Act states that no person shall sell an article of food that has in or upon it any poisonous or harmful substance. Section 24(j) of the Act provides the necessary authority to exempt any food, drug, cosmetic or device from all or any of the provisions of the Act, and to describe the conditions of such exemption. It is under the authority of Section 24(j) of the Act that regulations for the establishment of tolerances have been made. Section B.15.002 is the specific section of our regulations dealing with tolerances and reads as follows: "Except as provided in these regulations, a food set out in Appendices IV and V containing in or upon it (a) any or all of the poisonous or harmful substances listed in Appendix IV and Appendix V in amounts not exceeding the quantities stated therein in parts per million (p.p.m.) for that food, as determined by an acceptable method, and (b) no other poisonous or harmful substance is hereby exempted from the provisions of paragraph (a) of Section 4 of the Act."

Briefly stated, if a tolerance for a specific pesticide is not mentioned in these appendices the amount of residue permitted for it is zero. This is the interpretation placed on this Section by our Legal Department, and it seems to be one which is not too well understood. It is not necessary for us to publish a list of those pesticides for which the tolerance is zero. It should be understood by everyone marketing these products in Canada that until a tolerance is set for an agricultural chemical, the tolerance for it is zero, and anything else is in violation and subject to prosecution. Authority is provided in the Act to take the necessary action to confiscate food which contains residues in excess of the permitted tolerance.

I mentioned previously that we have had a number of tolerances in our regulations for a number of years. Most of these tolerances were set a number of years ago. When it became apparent that a number of tolerances would have to be set for the newer pesticides, the Technical Committee of the Canadian Agricultural Chemical Manufacturers Association was approached with the outline of a plan for the setting of tolerances for pesticides.

Several meetings were held and as a result, satisfactory requirements were worked out. Instead of issuing regulations for the requirements, it was thought that the best and easiest way to inform the Trade of our requirements was to issue what we call in the Food and Drug Directorate, a Trade Information Letter. These letters have no regulatory significance but they serve the purpose of informing the Trade of our views and interpretations placed on certain regulations. The particular T. I. L. dealing with "Tolerances for Poisonous or Harmful Residues in or on Foods" is 124 and reads as follows:

"Any person may file with the Director of the Food and Drug Directorate, a request that a tolerance be established for an agricultural chemical. He must, at the same time, show justification for the establishment of a tolerance, and present evidence that the use of the product is practical and that it is effective for the use recommended.

With each request the following information about the agricultural chemical must be supplied:—

- (a) The chemical name, the trade name and where applicable: the purity, stability, solubility, melting point, vapour pressure and density;
- (b) the amounts to be applied and the frequency and time of application;
- (c) full reports of investigations made to determine the safe levels (these reports are to include, where necessary, data and detailed information obtained from appropriate animal or other biological experiments in which the methods used and the results obtained are clearly set forth);
- (d) the results of tests on the amount of residue remaining in or on the food crop and the description of a satisfactory analytical method for determining residues in or on the foods or classes of foods for which it is recommended; and
- (e) a proposed tolerance.

Correspondence requesting the establishment of a tolerance for an agricultural chemical in or on a food crop should be addressed to the Director of the Food and Drug Directorate, of the Department of National Health and Welfare, Ottawa. It would be advisable to submit at least two copies of the information required by items (a) to (e) inclusive.

The information should be collected under the headings given above and should be as complete as possible. When experimental work is reported, a description of the methods used, the number of each species of animal used in biological tests, a description of the statistical design and analysis of such tests, and the results and conclusions drawn should be stated.

If the data and information are not considered complete or adequate, the applicant will be notified and some indication will be given of the kind of additional information required."

These requirements are essentially the same as required in the United States for setting of tolerances. It should be noted that, although we are not restricted to the setting of tolerances for fruits and vegetables, it was for this purpose that these requirements were drawn up.

There are two requirements about which some companies have been in doubt. I would like to elaborate on these briefly. These are the residue and toxicological data which must be submitted with each application for a tolerance.

#### RESIDUE INFORMATION REQUIRED

Before a tolerance can be established for a residue on food crops, well documented residue data for the particular pesticide should be presented.

Essential information should be presented as follows:

1. List the source of each sample, including the cooperator and the location from which the sample was obtained.
2. Describe how the sample was collected, e.g., one way of selecting a sample of apples is to take one apple from each of the four quarters of each tree, and to repeat this on about 10 trees supposedly treated with pesticide in exactly the same way.
3. Give the form in which the pesticide was used and the method of application.

4. Calculate the dosage in terms of 100 per cent pesticide per acre or other unit.
5. List the interval from treatment to sampling and also that from sampling to analysis. This is particularly important with unstable or volatile chemicals.
6. When the sample was not analyzed soon after collection, mention the conditions of storage.
7. Include a detailed description of the analytical methods used.
8. Describe the preparation of the sample for analysis. If any washings or brushings were discarded before extraction for analysis, this should be mentioned. With root crops describe the cleaning process. With foods which have a distinct peel, it is recommended that several samples of peel be analyzed, and the results reported in terms of peel alone as well as in terms of the whole food.
9. Preferably analyze one series of samples by surface extraction and another by extraction of the thoroughly macerated material to get not only the spray that has remained on the surface, but also that which has penetrated below the surface.
10. It is desirable to run controlled tests using the recommended rate of application. Analyses of the product at regular intervals should be made until the residue has diminished to a negligible value. This gives information on the rate of disappearance of the insecticide.

#### PRINCIPLES BEHIND THE GROUPING OF RAW AGRICULTURAL PRODUCTS WITH RESPECT TO PESTICIDE RESIDUE DATA

It is possible, in some cases, to predict that residue data on one growing crop represent residues likely to occur on another crop or other crops. Under such circumstances, it is reasonable to consider that all such crops should be in one group and regarded as one raw agricultural commodity.

The principles to be followed in grouping crops are:

- (A) For pesticides which are not systemics, crops which are members of the same botanical family may be grouped, provided:
  - (1) They have essentially the same type of plants and growth habits;
  - (2) The physical characteristics of the edible portions of the crops are essentially the same;
  - (3) Methods of applying pesticides to and harvesting the crops are similar.
- (B) Crops which are not in the same botanical family may be grouped where the other criteria under A above are met.
- (C) Crops in the same botanical family will not be grouped if the edible portions of the plants differ in characteristics which have an important bearing in the quantity of the pesticide residue which remains.
- (D) These principles do not apply in the case of pesticides which are systemics.

For example, apples, crabapples, pears and quinces would be grouped together. Also carrots, garden beets, sugar beets, horseradish, parsnips, radishes, rutabagas, salsify roots and turnips would be grouped together. These are just two examples. There are many others.

#### RESIDUES FOR TWO OR MORE CHEMICALS IN THE SAME CLASS PRESENT ON THE SAME CROP

We believe that it is reasonable to expect that if several pesticides in the same class appear on the same crop, the combined tolerance should not be the sum-total of the separate tolerances but be related to one of the tolerances in the group, preferably the most toxic. The Food and Drug Administration in the United States and the National Agricultural Chemical Association have worked out a suitable scheme. It was published in the Federal Register, April 14, 1956. At the present time we have not been able to improve on this method, and for the present we are willing to accept it.

#### ZERO TOLERANCES

We have been asked from time to time what we mean by zero tolerance. I believe that most of you recognize that it is practically impossible to demonstrate with certainty absolute

zero concentration of anything. We believe the logical approach to this problem is to consider what we might term as *threshold values* for pesticides in foods. These are levels below which there would seem to be no real pharmacological significance for a pesticide or groups of pesticides.

A fair rule of thumb might be that a minimum pharmacologically significant level for many of the pesticides such as aldrin, dieldrin, endrin, isodrin, chlordane, heptachlor and others, would be as low as 0.01 p.p.m. That is to say, one would be justified in regarding more than 0.01 p.p.m. as a level in excess of the practical equivalent of zero.

In a less toxic category are such pesticides as methoxychlor and the like, which would ordinarily certainly seem to lack real significance until present in levels as high as 0.1 p.p.m.

The establishment of *threshold values* would depend on whether satisfactory methods of analysis are available. There would not likely be any use of considering zero as 0.01 p.p.m. if the method was only sensitive to  $\pm 0.1$  p.p.m. In such cases it is questionable whether the product should be used on food crops at all.

I would not like to imply that we have adopted the scheme I have outlined, but we are considering such a scheme, and are continuing to carefully examine each problem that comes up in this connection, on the basis of the circumstances that pertain specifically to it.

#### TOXICOLOGICAL AND PHARMACOLOGICAL DATA REQUIRED

There have been a number of publications on procedures for the appraisal of the toxicity of chemicals in foods. Possibly the best known is the one which has been published by the staff of the Pharmacology Division of the Food and Drug Administration in Washington. This paper appeared in the Food, Drug, Cosmetic Law Journal, Oct. 1955. These procedures have been adopted by most of the manufacturers of agricultural chemicals in the United States and Canada. In brief these procedures include the following areas of investigation: 1. Acute Toxicity, 2. Pharmacodynamics, 3. Biochemistry, 4. Subacute Toxicity, 5. Chronic Toxicity, 6. Dermal Toxicity, and 7. Pathology.

It is necessary that investigations be carried out in these respective fields, and that adequate data be presented before a proper appraisal of the toxicity can be made and a tolerance set. The toxicity evidence, particularly that with respect to chronic toxicity, is evaluated to gauge the maximum safe level of residue. In this the principles adopted are the same as those adopted by the National Research Council in the United States and are contained in a report on Safe Use of Chemical Additives in Foods, published in Dec. 1952.

#### TOLERANCES AND THE PEST CONTROL PRODUCTS ACT

In Canada, before a pesticide can be marketed it must be registered. The registration of these products is the responsibility of the Pest Control Products Division of the Federal Department of Agriculture. We have been asked from time to time whether tolerances should be established at the same time as an application is filed for registration. We believe that the administrators of the Pest Control Products Act should be in a position to advise a registrant on the basis of the recommended conditions of use and the residue data submitted whether a tolerance should be set before the product is registered. If the product is going to be used in such a way that the amount of residue at harvest is nil, there is no necessity to have a tolerance established. As a matter of fact, it is the policy of the Food and Drug Directorate to not set tolerances if they are not needed. There is a very close liaison maintained between our Food and Drug officials and those of the Pest Control Products Division, and problems of mutual concern are discussed quite freely before decisions are made.

#### PROCEDURE FOR THE HANDLING OF APPLICATIONS FOR TOLERANCES

It is pointed out in the opening paragraph of Trade Information Letter No. 124 that a justification for the establishment of a tolerance must be shown and evidence must be presented that the use of the product is practical and that it is effective for the use recommended. It is quite apparent that it is beyond the scope of work of the Food and Drug Directorate to assess these reports in the application, and we rely on the advice of officers of the Federal Department of Agriculture after they have reviewed the data.

Following the receipt of a favourable report from the Department of Agriculture, the data in the submission are examined by various members of the Food and Drug Staff. This review is made up of two parts:



1) The toxicological and pharmacological data is reviewed in order to determine if the proposed tolerance is safe.

2) If the proposed tolerance appears to be safe, the analytical method and residue reports are examined. In this review we are particularly interested in knowing the precision of the method, and if the method will detect the amount of pesticide present when other interfering substances exist. The residues reported in the data are plotted and the rate of decay or degradation is mapped out. It is particularly important to know if the proposed residue tolerance is likely to be exceeded if the proposed rates of application and conditions of use are adhered to.

Assuming that the data is all in order and the proposed tolerance is agreed to, the manufacturer concerned is notified of our findings. The manufacturer has 30 days in which to state whether he agrees with the decision of the Directorate. If the tolerance is acceptable, a recommendation is made to the Minister of the Department of National Health and Welfare for a revision of the Food and Drug Regulations and, with his concurrence, a regulation is prepared for submission to the Governor-in-Council, and in due course it is published in the Canada Gazette and becomes part of Section B.15.002 of the Food and Drug Regulations.

Trade Information Letters will be issued periodically, showing the tolerances which have been set and the crops on which the tolerances may be applied.

Some of you may have observed that there are no provisions in our regulations for an Appeal Board. We have not considered that this was necessary. It is fairly well known that under our system of government in Canada, an individual always may appeal his case to the Minister of the particular Department involved and a hearing will be arranged.

#### TEMPORARY VERSUS PERMANENT TOLERANCES

No provision has been made in our regulations for setting temporary tolerances. I believe the Miller Bill in the United States provides for the setting of temporary tolerances. Our Food and Drugs Act, as it is presently constituted, does not provide us with this authority. At the present time there does not appear to be a real necessity for establishing temporary tolerances in Canada. At least, we have not had a specific request for temporary tolerances. If it is the wish of a particular company to have a temporary tolerance set and sufficient residue and toxicological data are presented, as well as compliance with the other requirements in T.I.L. No. 124, we will be glad to consider the setting of a temporary tolerance. It might be quite different from the tolerance that would be set if complete toxicological data were available, but it is our belief that, in most cases, we could set one which would permit agriculturists to use the pesticide during part of the experimental period so that the produce could be sold.

#### EXEMPTION FROM TOLERANCES

No provision has been made in our regulations to exempt a pesticide from a tolerance because of low toxicity or for other reasons. We believe that if a residue is present at harvest, even though its toxicity may be low, a tolerance should be set for it. It would appear that if certain pesticides are exempted from tolerances it might lead to indiscriminate use, and it is our belief that a residue tolerance must be set in order to retain control of the amounts which may be present on food.

#### COOPERATION WITH OTHER AGENCIES ENGAGED IN THE ESTABLISHMENT OF TOLERANCES

The officers of the Food and Drug Directorate are willing to cooperate at any time in discussions which would lead to the establishment of safe tolerances for pesticides. There seems to be a need for the pooling of information on the toxicological work and other related work which is presently being carried on in various countries. I suppose this can best be done through the appropriate committee of the World Health Organization. I am not in a position to say just how active this group is at the present time, but it is my impression that this international body meets too infrequently to really keep everyone up to date with what is going on in other parts of the world in this particular field. An organization which would receive information for distribution to other centres in the world engaged in the business of setting of tolerances would be helpful. It is a real problem for manufacturers and distributors of agricultural chemicals to have all the necessary investigations carried out before an appraisal of the toxicological properties can be made. A good deal of the work which is done in the United States and Canada at times is a duplication of work that has

already been done in England and continental Europe. It would seem to us that, if the methods for toxicological appraisal were the same, some of this duplication could be eliminated.

I know at the present time there are some people who are of the opinion that existing toxicological requirements are too strict. The time has possibly come for closer cooperation on an international level on methods for appraising the toxicological aspects of these pesticides. At a recent meeting of a W.H.O. Study Group on the toxic hazards of pesticides to man, some thought was given to this problem, but no definite action was taken.

#### TOLERANCES SET SINCE MAY 1955

Since the issuance of T.I.L. No. 124 we have received 13 applications for tolerances and one exemption from a tolerance. These were for: Malathion, Parathion, Sulphenone, Captan, Sodium-o-Phenylphenate, Piperonyl Butoxide, Piperonyl Butoxide and Pyrethrins, DDT, Diazinon, Aramite, Allethrin, Ovotran, Methyl Bromide and Ethylene Dibromide. Tolerances have been set for Malathion, Parathion, Captan, Aramite and Sodium-o-Phenylphenate, and regulations showing the particular crops on which the tolerance may be applied have been published in the Canada Gazette.

#### CONSERVATISM IN THE USE OF PESTICIDES

The Food and Drug Directorate in Canada has long voiced the opinion that conservatism should be exercised in the use of pesticides, particularly if food is liable to be contaminated. We have never been among those who would have people believe that our food supplies are being exposed excessively to pesticides, and relate these exposures to unexplained illness. On the other hand, we have never believed in the theory that dangers from spray residues in food are greatly overemphasized. The fact that no person has been killed or made seriously ill by eating foods which have been treated with, or exposed to, a pesticidal chemical at some stage in its production seems like a poor argument for indiscriminate use.

Publicizing of statements to the effect that no one has yet been made seriously ill by consuming foods that may have been contaminated with small amounts is a great disservice to the public. The demonstration and recognition of acute symptoms, leading to death or serious injury, do not enter this argument at all, since we are dealing with chronic effects. We believe what should be emphasized is that sub-chemical disease entities, whether aroused by pesticidal residues or any other chemical insult to the body, are so difficult to recognize that even the expert may have difficulty in correlating the facts. A more conservative view somewhere between the two extremes, that the public is in serious danger from the ingestion of small amounts of pesticides in foods, and that pesticidal residues are entirely harmless, would seem to be the best course. Although all known evidence indicates that there is no immediate danger to the public health, measures should be adopted to insure that a dangerous situation does not materialize. Possibly more time should be spent in stressing the need to follow the recommended directions for use.

We do our best in screening these chemicals for possible harmful effects, but we cannot be absolutely certain that the residues ingested by man are harmless; there is no data that proves this even beyond a reasonable doubt. It would appear to us that conservatism should govern all our actions when dealing with the safety aspects of pesticides at the present time. By this we do not mean that unnecessary restrictions should be placed on the use of pesticides below limits which will serve a useful purpose, but we do mean that relaxation of controls is not justified on the basis of present scientific knowledge.

#### DISCUSSION

J. A. FREEMAN. Do insecticide tolerances apply to export commodities, especially grain and flour?

M. G. ALLMARK. The Canadian Food and Drugs Act does not apply to any packaged food, drug, cosmetic or device not manufactured for consumption in Canada and not sold for consumption in Canada, if the package is marked in distinct overprinting with the word "Export", and a certificate that the package and its contents do not contravene any known requirement of the law of the country to which it is or is about to be consigned, has been issued in respect thereof in prescribed form and manner.

J. A. FREEMAN. Explain the difference in treatment of stored grain and growing crops with regard to setting of tolerances, especially for pyrethrins and piperonyl butoxide.

M. G. ALLMARK. In Canada there has been no difference in the treatment of stored grain and growing crops with regard to setting of tolerances. If there is a need to establish a tolerance, one should be set

J. A. FREEMAN. To what extent are imports sampled chemically to see whether they comply with tolerances?

M. G. ALLMARK. Our analysts located in several centres across Canada spot-check imported foods to see if the tolerances are exceeded.





# Heptachlor: A Review of its Use as an Insecticide<sup>1</sup>

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## ABSTRACT

Heptachlor is one of the effective chlorinated hydrocarbon insecticides available today for control of a large number of important insect pests.

As an insecticide, it has been widely tested against a variety of insect pests in many parts of the world. It is effective and economical for the control of many soil inhabiting species, including rootworms, root weevils, white grubs, root maggots, wireworms, white fringed beetles and others. Heptachlor has given good control of the cotton boll weevil and cotton thrips. The alfalfa weevil, the alfalfa snout beetle, spittlebugs, the Egyptian alfalfa weevil, sweet clover weevil and several other legume pests are controlled by heptachlor insecticides. Ants in a variety of situations are effectively controlled. Grasshoppers, European corn-borer, cutworms, and a number of other pests have been discussed with reference to control with heptachlor.

In the field of insects annoying to man and animals, heptachlor has proved effective for mosquito control as well as for sand flies and eye gnats. It has shown promise in testing work with some species of lice and ticks.

A tolerance of 0.1 p.p.m. has been established for heptachlor residues by the Food and Drug Administration for a number of raw agricultural commodities. This provides a satisfactory working level for heptachlor insecticides approved for use on these commodities. A sensitive, reliable analytical method makes accurate residue determination possible. Bio-assay methods have been used by various experimentors. There has been no phytotoxicity and no problem with off-flavors from the use of heptachlor insecticides when label directions for their use are followed.

Heptachlor, first reported in 1949 by Kearns *et al.*, has been used extensively in field control programs for the control of a variety of insect pests, and included in research programs in all parts of the world for evaluation in the control of other species. It is obvious from the literature and field experience of the past seven years that heptachlor in most instances is one of the more effective chlorinated hydrocarbon insecticides that can be selected for particular control measures against a given pest. For many uses it compares favorably with aldrin, dieldrin, DDT, BHC, lindane, endrin and a number of phosphate compounds, and as such, it is recommended by government agencies as one of the insecticides that will provide effective control in specific recommendations. Depending upon the conditions of the experiments, the species of insects involved, the formulation and the like, heptachlor has performed insecticidally in a manner to justify equal consideration with other effective materials. In some instances, it has not. This is true, however, with all insecticides.

The purpose of this discussion is to review some of the areas in insect control where heptachlor has shown good performance as an insecticide, and to indicate some of the fields of research where it is under trial in laboratory and field experimentation. It is to be emphasized that in this discussion, the failure to make direct efficiency comparisons with all of the other insecticides included in the published research or recommendations, is not an attempt to disregard the efficiency of the other insecticides. Rather, it is thought that by selecting areas of pest control where heptachlor is currently recommended, or is showing good promise, a clearer picture can be given of its performance during the years since its introduction as an insecticide.<sup>2</sup>

## INSECTICIDAL EFFECTIVENESS

Heptachlor has given excellent control of many soil inhabiting insect pests, including several species of rootworms, wireworms, root maggots, root weevils, white grubs, grasshoppers and the like.

<sup>1</sup> Directions for formulation of heptachlor dusts, wettable powders, emulsifiable concentrates, oil solutions, granular formulations, fertilizer heptachlor mixes, are provided by the Velsicol Chemical Corporation.

<sup>2</sup> References, prepared for distribution, are available upon request.

The southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, causes extensive damage to peanuts in its larval feeding stage. In the southern part of the United States, heptachlor, among other materials, has given excellent control of this pest. Among the several methods of control recommended 1-1/2 to 2 pounds of actual heptachlor per acre give economical control when applied prior to planting, just before first cultivation or during the early blooming period. Ritcher *et al.* reporting on several insecticides for the control of *D. howardi* on peanuts indicated that heptachlor at 1 or 2 lbs. per acre gave excellent control. Gilpin *et al.* conducted palatability tests with peanuts grown in Virginia soil treated with several insecticides for rootworm control. On the basis of flavor evaluation, insecticidal efficiency and cost, soil applications of 2 pounds per acre of heptachlor were included in the recommendations for control of the southern corn rootworm on peanuts.

Heptachlor has proved itself as a valuable soil insecticide for use in control of corn rootworms. Cox and Lilly indicate that among the various materials tested for *Diabrotica longicornis* Say in Iowa, heptachlor provided good control at the rate of 8 ounces per acre when applied in a band over the planter trench with a planter-mounted sprayer. Lilly reported extensive experiments with a series of chlorinated hydrocarbon insecticides, applied to the soil by a number of different methods. Among the effective insecticides in rootworm control, heptachlor appeared equally effective, pound for pound, with other materials tested. Bigger and Blanchard working with the control of the more injurious insects found in corn fields in Illinois, reported heptachlor as one of the effective insecticides. A dosage of 1.5 pounds per acre controlled moderate soil insect infestations. One pound was sufficient for northern corn rootworm, but not for all other insects. It was important to work the insecticide into the soil immediately after application, regardless of the manner of treatment, whether broadcast, spray or fertilizer mix. Ball records heptachlor as being one of the insecticides widely used for the control of several species of corn rootworms *Diabrotica* spp. The experiments reviewed were designed to see the effect of three insecticides used for corn rootworm control on the growth of corn roots. Heptachlor treatments resulted in plants having roots that compared favorably with the controls.

The increasing spread of the European corn borer, *Pyrausta nubilalis* (Hbn.), into the mid-western corn belt and its southern movement in the United States has caused increased concern over its control. An interesting development in research to control the borer is reported by Cox *et al.* through the use of granular insecticides as compared with emulsion sprays. The granular formulations on tobacco-base and attapulgitic carriers were applied by modified grass seeders and a power duster. One and one-half to 2 pounds of heptachlor applied as 7-1/2% and 10% granular formulations under conditions of the experiments in 1953 and 1954 gave corn borer control comparable to the best of the other insecticides in the experiments. The authors point out that residues of DDT on sprayed leaves were up to 200 times more than residues from granular applications; sprayed plants had about twice as much residue in the leaf axil and stalk area. They further conclude that granulated formulations of insecticides are just as effective, and perhaps more effective, than emulsion sprays for control of the European corn borer.

### GRASSHOPPER CONTROL

Grasshoppers are injurious to range grass, forage, grain and other crops. Heptachlor has been effective as spray emulsions or in dusts and baits. Direct treatment of the crop at recommended levels can be carried out up to 7 days before harvesting or pasturing without leaving harmful residues. Two ounces of actual heptachlor are sufficient per acre for the control of nymphal grasshoppers. In tall, dense foliage and with large nymphs and adults, 2 to 4 ounces per acre are recommended. Barnes *et al.* indicated heptachlor gave satisfactory kills of grasshoppers in Arizona and Montana in both spray and bait formulae. In Argentina, heptachlor has given excellent control of "tucura", a species of grasshopper.

### ROOT MAGGOTS

Heptachlor is one of the effective insecticides for control of the cabbage maggot, *Hylemya brassicae* (Bouche). Davis *et al.* report effective control with broadcast applications of heptachlor at approximately 3 to 4 pounds per acre prior to planting. Use of heptachlor at 2 ounces per 50 gallons of transplant water or 1 lb. per acre in the setting furrow have given satisfactory control on cabbage transplants. Forbes and King report the control of

root maggots in rutabagas in British Columbia. The species primarily concerned was *H. brassicae*. Heptachlor spray and dust formulations applied to the rows were among the recommended materials for control. Stitt reported heptachlor dust as a furrow treatment as one of the insecticides that effectively controlled the cabbage maggot, *H. brassicae* on turnips in western Washington, without off-flavor or excessive residues. Morrison and Crowell list heptachlor as a promising all-purpose soil insecticide for control of cabbage maggots, seed corn maggots, onion maggot and carrot rust fly.

The onion maggot, *Hylemya antiqua*, was reported controlled by combining heptachlor, as one of the effective insecticides, with the fungicide, thiram, as a blow-in treatment in the seed furrow. Other recommendations include seed treatment, drench and blow-in furrow treatment. Peterson and Noetzel reported several insecticides including heptachlor effective as seed pelleting, when applied as a mixture with a fungicide.

Jones *et al.* reported the control of the sugar-beet maggot, *Tetanops myopaeformis*, by the use of soil and seed treatments with heptachlor. Heptachlor was listed as one of the effective materials used.

Antonelli and Ciampolini report excellent control of cleono, *Cleonus mendicus*, on sugar beets in the Po Valley of Italy either as a foliage spray or soil treatment.

The carrot rust fly, *Psila rosae* (Fabricius), is controlled by application of heptachlor according to approved methods in Holland as well as North America.

The narcissus bulb fly, *Lampetia equestris*, is a serious pest of narcissus bulbs during the larval stage. Doucette, based on laboratory and field experiments, indicated heptachlor proved to be one of the most effective materials for pre-planting immersion treatments. Heptachlor at 0.5 pound per 64 gallons of water, with a soaking period of 10 minutes, gave complete control of the bulb fly without any damage to the bulbs. This is also confirmed in Holland.

Rui reports two years practical results in Italy with heptachlor as a soil treatment only, giving good control of *Rhagoletis cerasi*, the cherry fruit fly.

### ALFALFA WEEVIL

The alfalfa weevil, *Hypera postica*, has been a serious pest of alfalfa since its introduction into western United States. Heptachlor has proved itself to be a most valuable insecticide in controlling this insect. Treatment for destruction of over-wintered weevils in the early spring can be obtained with 4 ounces of heptachlor per acre applied as a spray or 5 ounces applied as a dust. Applications as low as 3/4 of an ounce of heptachlor per acre have given control of the larvae on alfalfa and can be used up to 3 days before harvest. Ten days should intervene between application of the 4 ounce per acre rate and feeding of treated alfalfa to milk cows. Early spring applications are made before pollinating bees are present in the fields and in the western states, no injury is caused to weevil parasites.

### OTHER ALFALFA AND CLOVER PESTS

Gyrisco *et al.* in reviewing experiments with several chlorinated hydrocarbon insecticides in laboratory and field experiments for control of the alfalfa snout beetle, *Brachyrhinus ligustici* L., when incorporated in baits for the adult beetles, reported heptachlor as one of the most effective toxicants. One ounce of heptachlor per acre of baited alfalfa has given excellent control. This species is a serious pest of alfalfa in Central Europe.

Reynolds *et al.* review the work relating to the Egyptian alfalfa weevil, *Hypera brunneipennis* (Boh), since its first discovery in the United States in 1939. Heptachlor was shown to be one of the insecticides that gave excellent control of the larvae at 1 ounce per acre. It also gave good control of the adults when applied at the rate of 0.45 pound per acre to stubble alfalfa to kill the adult weevils prior to oviposition.

Allen and Kelleher report effective field control of the sweet clover weevil, *Sitona cylindricollis* Fahr., with 1.0 pound of heptachlor per acre. Allen and Askew reporting on the protection of seedling crops against this pest in western Canada, found heptachlor spray applications at the rate of 0.5 pound per acre to be one of the most effective insecticides tested.

App in discussing insecticide treatment for the clover root borer, *Hylastinus obscurus* (Marsh), and the meadow spittlebug, *Philaenus leucophthalmus* (L.), reported several in-

secticides that gave excellent to good control of these species. Of these, heptachlor dust as a spring treatment at 1-1/2 to 1-3/4 per acre gave excellent control and also controlled nymphs of the meadow spittlebug.

In Turkey heptachlor dust is recommended for control of the filbert weevil, *Balaninus nukum*.

Melis in Italy has reported promising results with heptachlor as a soil treatment against the same insect which attacks chestnuts.

### ROOTWEEVILS

Weevils of the genus *Brachyrhinus*, *B. ovatus* and *B. sulcatus*, attack a variety of nursery crops and also are injurious pests of strawberry. Eide in reviewing several effective insecticides for use on strawberries to control these pests, reported heptachlor as one of the effective materials.

### CUCUMBER BEETLES

Michelbacher *et al.* in discussing the control of cucumber beetles *Diabrotica undecimpunctata* Mann and *Acalymma trivittata* (Mann.) on melons in California listed heptachlor as one of the insecticides.

### ONION THRIPS

The control of onion thrips, *Thrips tabaci* Lind., on onions has been successful in several areas of the United States through the use of heptachlor dusts and sprays. Applications should not be made closer than 5 days of harvest. Sloan and Rawlins compared a number of insecticides, including different formulations, for onion thrips control. Heptachlor proved to be effective under the conditions of their experiments.

### WHITE GRUBS

Howe and Campbell discuss results of field experiments in the control of the green June beetle, *Cotinus nitida* (L.), as a pest of Ladino clover in Virginia. A number of effective materials for the control of this pest were listed. Heptachlor at 2 pounds per acre was shown to be highly effective when applied shortly before the eggs hatched in late June or early July. Later applications against third instar larvae were ineffective.

Evans and Gyrisco report heptachlor in granular formulation at 1 and 2 pounds per acre of actual toxicant as giving excellent control of the European chafer, *Amphimallon majalis* (Raz.), for two successive generations. Burrage and Gyrisco had previously reported the effectiveness of heptachlor for the same species at the 2 pound dosage per acre, applied in dust form. Control in pasture sod was noted for three generations of larvae. Gambrell working with this species in lawn turf reported several effective soil treatments, including heptachlor at 2.5 pounds per acre. Polivka used heptachlor for turf inhabiting insects in Ohio. Heptachlor at all levels in the experiment, ranging from 1 to 20 pounds per acre, gave control of grubs of the Japanese beetle, *Popillia japonica*, the annual white grub, *Cyclocephala borealis*, and white grubs, *Phyllophaga* spp.

### WIREWORMS

Heptachlor has been used to good advantage to provide wireworm control on a variety of crops. Rawlins *et al.* in reporting on experiments for control of the eastern field wireworm, *Limonious agonus* (Say), and the wheat wireworm, *Agriotes mancus* (Say), on potatoes, found heptachlor gave good control at 2 to 4 pounds per acre. Morrison and Crowell conducted extensive experiments in Oregon for the control of several soil insect pests, including wireworms. Of the effective material reported, heptachlor, based on limited tests, was rated as a promising all-purpose soil insecticide. Griffin and Eden working with the Gulf wireworm, *Conoderus amplicollis* (Gyll.), as a pest of sweet potatoes, reported heptachlor as one of the insecticides giving good wireworm control over the two-year test period. Kulash conducted grower trials for control of wireworms attacking corn in eastern North Carolina. The principal species involved was *Melanotus communis* (Gyll.). Reporting on the several insecticides in these tests, heptachlor showed good effectiveness. Further field studies reported by Kulash and Monroe found heptachlor effective. Hyche and Eden studied wireworm control in sweet potatoes and indicated that heptachlor was one of the effective insecticides in the experiments. Rabb *et al.*, reporting on the use of heptachlor for wireworm control in transplant water in the flue-cured tobacco belt of North



Carolina, found that the emulsifiable formulation gave better control than wettable powders. Guthrie and Rabb reporting on broadcast applications of soil insecticides as well as soil fumigants for wireworm control on tobacco, state that heptachlor was one of the insecticides that gave excellent control of heavy infestations of wireworms in test plots. Lhoste in Brittany, France reports outstanding control of wireworms using heptachlor dust in potatoes and corn. Similar results have been reported from Italy and Turkey.

### ANTS

The western harvester ant, *Pogonomyrmex occidentalis* (Cresson), was found to be controlled satisfactorily by the use of heptachlor applied to the entrance of the nests. Knowlton and Baldwin report excellent control of *P. occidentalis* and eight other species of ants with heptachlor dust concentrations from 1 to 25%. The silky ant, *Formica fusca*, and the European earwig, *Forficula auricularia*, in gardens and lawns were controlled with similar treatments. Carman reported heptachlor as one of the effective insecticides for the control of the Argentine ant, *Iridomyrmex humilis*, when applied at the minimum rate of 2.5 pounds of actual toxicant per acre. Srivastava and Bryson report on the treatment of sorghum seed in south-central Kansas to protect it from destruction by the thief ant, *Solenopsis molesta* (Say). Heptachlor at the rate of 5.0 ounces per 100 pounds of seed was one of the effective insecticides used in the experiment. Workers in Costa Rica and Argentina report particularly good control of leaf-cutting ants, *Atta* spp.

### COTTON INSECTS

Heptachlor alone and in formulation with other insecticides gives good control of cotton insects in the southern United States, including the cotton boll weevil, thrips and several species of plant bugs. Other materials are added to formulations to control bollworms, leafworms, aphids and spider mites as in general heptachlor is not effective against Lepidoptera nor aphids or red mites. The Cotton Insect Control Conference included heptachlor as one of the insecticides for cotton bollweevil control at the rate of 0.15—0.5 pound of active ingredient per acre.

### RICE LEAF MINER

Lange and Ingebretsen reviewed the work in California on control of the rice leaf miner, *Hydrellia griseola* var. *scapularis* Loew. These workers reported that a combination of water level management and insecticide application proved effective in bringing the pest under control. Heptachlor applied by plane at 1/2 pound of toxicant in 10 gallons of water per acre, gave excellent control of the larvae and adults.

### BEAN WEEVILS

Ruppel found heptachlor effective in killing bean weevils, *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boh.) in experiments with stored beans in Colombia.

### POTATO FLEA BEETLE

Gould and McCrosky reported heptachlor as being effective for potato flea beetle control at the rate of 0.75 pound per acre per application. Five applications were made.

The Colorado potato beetle is effectively controlled by heptachlor sprays containing 0.5 pound per 100 gallons of spray according to studies in New York.

### WHITE FRINGED BEETLE AND CUTWORMS

Heptachlor insecticides have proved effective in the control of the white fringed beetle and several species of cutworms and armyworms. Applications have been made by dusts, sprays and granular formulations for cutworm control.

### BANANA PESTS

Thornton has reported heptachlor as one of the effective insecticides that will kill the adults of the banana borer, *Cosmopolites sordidus*, in Central America. Heptachlor has been shown to be promising in the control of the red rust thrips, *Chaetanophothrips orchidii* (Moulton), on bananas.

### MOSQUITOES

Hoffman reported on a series of larvicide experiments in Oregon. Heptachlor applied at 0.025 pound per acre as emulsions, oils, and granules for control of *Aedes* in snow-water and flood complexes, gave mortalities, based on 24 hour counts, equal to DDT.

### FLEAS

Ryckman *et al.* in reporting on several chlorinated hydrocarbon insecticides for the control of fleas that infest ground squirrels in California, found heptachlor to be one of the best insecticides tested under field conditions. Smith also reported heptachlor as effective among the insecticides he used in laboratory tests with the cat flea and the oriental rat flea.

### EYE GNATS

Tinkham reported on several soil insecticides that were disked into the soil after spraying on the surface at the rate of 2-3 pounds per acre. Among the more effective in reducing gnats, primarily *Hippelates collusor*, was heptachlor.

### SAND FLIES

Goulding *et al.* reported on experiments for control of salt-marsh sand flies in Florida. Heptachlor was found to be one of the effective insecticides, applied at the rate of 0.25 pound per acre on small plots.

### BLACK FLIES

Hocking tested a series of insecticides against larvae and pupae of the black fly, *Simulium venustum*, in streams near Churchill, Manitoba in 1951 and 1952. Heptachlor gave excellent control at concentrations as low as 0.5 p.p.m.

### TICKS

Logg and Shanahan conducted experiments with several insecticides for control of the cattle tick, *Boophilus microplus*, that were resistant to sprays containing 0.2% arsenic. Among other effective materials, heptachlor as a 0.2% spray was listed.

### LICE

Blanton working with the human body louse, *Pediculus humanus* DeG., screened a series of insecticides in the laboratory to determine their toxicity to this species of louse. Heptachlor was reported as superior to DDT at 50 p.p.m., and gave 100% control at 6.25 p.p.m.

# Some Effects of Insecticides on the Wireworms and Vegetation of Grassland in Nova Scotia<sup>1</sup>

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## ABSTRACT

Experiments were conducted on grassland in four localities in Nova Scotia from 1952 to 1956 to evaluate the effectiveness of aldrin, chlordane and lindane in controlling the wireworms *Agriotes mancus* (Say), *A. sputator* (L.), *A. lineatus* (L.) and *A. obscurus* (L.). The insecticides were applied as surface sprays without subsequent cultivation. All treatments resulted in increased yield and quality of hay and in reduction of weeds. Lindane caused high mortality quickly but re-population by wireworms of soil treated with this insecticide apparently began 28 months after application. Aldrin and chlordane caused high mortality more slowly and re-population was not observed until the fourth year after application.

In a separate experiment it was observed that aldrin, dieldrin and heptachlor applied in the same way controlled *Collembola* and *Acarina* to some extent.

## INTRODUCTION

Nova Scotia is plagued with several injurious species of wireworms in her arable lands. Three troublesome European species of *Agriotes* have augmented the native population and have become established in several coastal areas. For many years they were thought to be the native wheat wireworm, *A. mancus*, until Brown (1940) reported their identity as *A. lineatus*, *A. obscurus*, and *A. sputator*. The distribution of these exotic species in Canada was recently reported by Eidt (1953).

Following observations that pasture and haylands were "running out" or deteriorating in yield and quality probably because of wireworm infestations, experiments were begun in 1952 to evaluate the effectiveness of aldrin, lindane and chlordane applied as surface sprays to grassland without subsequent cultivation to mix the insecticides with soil. By avoiding the complications arising from cultivation it was possible to observe the effects of the insecticides for several years in a physically undisturbed habitat.

## METHODS

The experiments were conducted at Hall's Harbour, Kings County, Chebogue, Yarmouth County, Cow Bay, Halifax County, and Digby, Digby County, in fields respectively infested with *A. mancus*, *A. lineatus*, *A. obscurus*, and *A. sputator*. The experiment at Digby will be dealt with chiefly because the results were more precise in consequence of the extremely high wireworm numbers—about 2,000,000 larvae per acre. In the other experiments the results were similar but less definite.

Latin square, split-plot designs, consisting of four or five treatments were used. Each plot was 25' x 30', one-half of which was left as an untreated check.

A single treatment was given, in the spring of 1952, using the following insecticide formulations applied as sprays at the rate of 116 gallons of water per acre: aldrin 20 per cent emulsible concentrate<sup>3</sup> at 3 and 6 pounds of toxicant per acre, lindane 25 per cent wettable powder<sup>4</sup> at 0.75, 1.0 and 1.5 pounds of toxicant per acre, and chlordane 40 per cent wettable powder<sup>5</sup> at 8 and 10 pounds of toxicant per acre.

To determine the effect of the treatments on the population of elaterids, carabids and staphylinids, samples of soil six inches square and eight inches deep were taken at random from each sub-plot at approximately monthly intervals, except during January, February and March. The marginal two feet of the plots, however, were avoided in sampling. Depending on its moisture content, the soil was sifted through either one-quarter inch or one-sixth inch mesh wire screen to recover the insects.

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<sup>2</sup> Associate Entomologist.

<sup>3</sup> Shell Chemical Company of Canada, Ltd., Toronto, Ont.

<sup>4</sup> Green Cross Insecticides, Montreal, Que.

<sup>5</sup> Dow Chemical Co., Midland, Mich.

To determine the effects of the treatments on the composition of the vegetation, two samples, each a quadrat 20 centimetres square, were taken at random in each sub-plot in September in 1954 and in late June in 1955 and 1956 and the percentage of surface at ground level covered by each species of plant was recorded.

To determine the effects of the treatments on yields, two square yard samples of hay were taken at random from each sub-plot. The hay was dehydrated to 5 — 1 per cent moisture content before weighing to level the loss of moisture in the different samples.

Dr. A. D. Baker, Officer-in-Charge, Nematode Investigations, Entomology Division, Ottawa, examined soil from plots at Digby treated with aldrin at 6 pounds per acre, chlordane at 8 pounds per acre and lindane at 1 pound per acre. His findings suggested that aldrin and chlordane had slightly lowered the nematode population but the data were not sufficient to give a good picture of what had occurred.

Grateful thanks are extended to Dr. I. V. Hall, Plant Pathology Laboratory, Kentville, N.S., for supervising the surveys of the vegetation.

## RESULTS

### EFFECTS ON SOIL FAUNA

The efficacy of the different treatments in controlling wireworms is shown in Table I which gives the average number of larvae per sample and the per cent control based on the adjacent check plots of each treatment for the years 1952–56. Treatments with aldrin and chlordane apparently required a year or more to approach full effectiveness, but maintained good control for three years after the insecticides were applied. Usually, lindane acted more quickly, reaching greatest effectiveness within eight months of application, but was not effective for as long as the other insecticides. Probably lindane is too volatile to be used economically as a surface spray.

The resurgence or “flare-up” of the wireworm population in plots at Digby treated with lindane at one pound per acre is unique; this phenomenon following initial control has not been reported previously among elaterids. The suggested explanation for this resurgence is that by the first spring after treatment lindane had so decreased the wireworm population that the grasses grew more abundantly and attracted ovipositing females leading to a decided increase in the larval population from 1954 to 1956. Possibly there was some movement of larvae from the poor sod around the plot margins to better pasture within the plots. Resurgence was also evident by 1956 in plots treated with aldrin at three pounds per acre. McColloch (1926) cites a somewhat parallel instance of a small elaterid, *Drasterius elegans* [= *Aeolus mellillus mellillus* (Say)], being attracted in large numbers to Sudan grass sown for pasture in Kansas.

In comparing the results from Hall's Harbour, where the treatments were applied in September, with the other experiments, there did not appear to be any difference in the effectiveness of spring or fall treatments, but obviously, if cropping were planned for the following year, treating in the fall would be advantageous as a measure of control would be obtained by spring. If spring treatments were followed by a dry summer, control might be seriously delayed, and the insecticides, especially lindane, might be weakened appreciably by volatilization.

In all but one location the data on larval and adult carabids and staphylinids were too meagre to have significance statistically, but suggested that by 1955 the populations of these forms in the treated plots had returned to normal. There was strong evidence in the data from Hall's Harbour that lindane was less harmful than aldrin or chlordane to carabids and staphylinids.

To evaluate the effects of surface applications of aldrin<sup>6</sup>, dieldrin<sup>6</sup> and heptachlor<sup>7</sup> on the populations of Collembola and Acarina, a series of four-ounce samples of turf with adhering roots and soil was taken in June, July and August, 1956, from plots treated in September, 1955. The samples were placed in modified Berlese funnels for 48 hours and the emerging soil fauna were collected in a solution of chloral hydrate placed below the funnels. At the rate of six pounds of toxicant per acre, the three insecticides effected measurable control. Apparently the mites in the soil were less susceptible to these chemicals than were the Collembola. (Table II).

<sup>6</sup> A 20 per cent emulsible concentrate provided by Shell Oil Company of Canada, Ltd., Toronto, Ont.

<sup>7</sup> A 20 per cent emulsible concentrate provided by Velsicol Chemical Corporation, Chicago, Ill.



TABLE I—Control of *A. sputator* with Soil Insecticides and the Effect on the Yield of Hay, Digby, N.S.

Insecticide (lb./ac.)	Wireworms per 0.25 sq. foot*					Per cent control**			Increase in hay yield (lb./ac.)			Per cent increase in yield**		
	1952	1953	1954	1955	1956	1952	1953	1954	1955	1956	1953	1954	1955	1956
Aldrin	6	2.4	1.2	.8	.7	71	85	90	90	82	1222	620	909	590
"	3	5.3	2.9	4.5	4.0	18	50	56	47	-14	720	529	464	547
Lindane	1	5.9	4.6	11.5	9.0	41	21	-25	-22	-55	744	212	315	197
"	0.75	4.8	5.4	9.5	7.9	33	14	13	10	-23	182	3	142	-27
Chlordane	8	3.3	2.5	3.8	2.4	54	58	61	68	0	684	348	325	227
L.S.D. 5 P.C.P.			2.3	2.0	2.3						793		501	242
In check plots†	8.5	6.5	9.8	7.9	3.8						1185	534	373	128

\*An average of 40 to 50 annual samples from treated split-plots.

\*\*Based on the mean values of five split-plot checks for each treatment. A minus sign preceding the value indicates either that the number of wire-worms in the treated samples exceeded that in the untreated or that the yield of hay in the untreated plot exceeded the yield in the treated.

†Based on the mean values of all split-plot checks; the values for hay are absolute yields.

TABLE III—Surveys in 1954, 1955, and 1956 of Vegetation on Treated and Check Plots Infested with *A. sputator*.  
Soil Insecticides Applied May 1, 1952. Digby, Nova Scotia

Species	Percentage of area occupied by various plants in treated and adjacent check plots											
	Aldrin 6 lb. per acre				Lindane 1 lb. per acre				Chlordane 8 lb. per acre			
	1954	1955	1956	1954	1955	1956	1954	1955	1954	1955	1956	1954
	Tr. Ck.	Tr. Ck.	Tr. Ck.	Tr. Ck.	Tr. Ck.	Tr. Ck.	Tr. Ck.	Tr. Ck.	Tr. Ck.	Tr. Ck.	Tr. Ck.	Tr. Ck.
Desirable												
Trifolium repens	3	2	5	4	18	2	14	4	4	2	5	12
Plantago lanceolata	25	1	12	0	15	2	13	2	8	0	0	11
Anthoxanthum odoratum	0	0	32	2	32	4	0	0	9	0	0	0
Trifolium pratense	6	1	9	0	8	1	5	7	3	1	9	2
Phleum pratense	19	5	5	2	0	0	4	2	5	0	0	7
Minor grasses	3	0	0	2	0	1	0	0	2	0	0	2
Total desirable	56	9	63	10	73	10	36	15	31	3	14	34
Undesirable												
Hieracium spp.	24	52	24	55	16	60	30	42	33	59	45	29
Daucus carota	11	30	5	18	2	9	19	17	13	17	11	6
Leontodon autumnalis	5	7	7	11	0	3	3	17	3	10	11	14
Minor composites	1	2	1	4	5	11	9	3	12	5	4	11
Total undesirable	41	91	37	88	23	83	61	79	61	91	71	60

TABLE II—Control of Collembola and Acarina on Plots Treated with Insecticides September 7, 1955. Digby, Nova Scotia.

Insecticide and rate per acre (lb.)		Collembola per sample* Tr.                      Ck.		Per cent control	Acarina per sample* Tr.                      Ck.		Per cent control
Aldrin	6	45	73	38	56	71	21
Dieldrin	6	37	62	40	51	57	11
Heptachlor	6	43	63	32	53	59	10

\*An average of 60 four-ounce samples of soil and turf taken through June, July and August, 1956 from each treatment.

#### EFFECT ON VEGETATION

Table III shows the effects of the insecticide treatments on the vegetation. For brevity, the lower rates of aldrin and lindane, several infrequent species of plants, and bare ground which was considered a unit, have been omitted from the table. The area covered by the desirable plants, the grasses and clovers, increased greatly in most of the treated sub-plots. Nearly all the weeds, especially the hawkweeds, *Hieracium* spp.,<sup>8</sup> *Taraxacum officinale* and *Leontodon autumnalis*, and wild carrot, *Daucus carota*, declined in numbers, presumably because they were dominated by grasses relieved of the drain on their vitality caused by the feeding of wireworms. A notable exception was ribgrass, *Plantago lanceolata*, which increased on treated soil. The increase in ribgrass may not be so undesirable as it first appears as it is readily grazed, is high in minerals, and may have tonic qualities (Moore, 1949). With the exception that ribgrass and wild carrot were not present, the effects at Cow Bay were similar.

These records indicate that the favored foods of *A. sputator* were grasses, clovers and ribgrass. It has been accepted frequently that clover is very tolerant of wireworm injury; the opposite appeared to be the case in this experiment.

#### EFFECT ON HAY YIELD

The increase in yield of hay in treated plots is shown in Table I. As was obvious in the standing crop, all treated sub-plots gave increased yields but those treated with aldrin were outstanding. Trials conducted in the laboratory greenhouse did not indicate that any of the insecticides stimulated the growth of grasses and clover, therefore the increase in yield is attributed chiefly to the control of wireworms.

#### CONCLUSION

Under Nova Scotian conditions, wireworms may be controlled conveniently by applying insecticides to the surface of grassland without subsequent cultivation. However, one application may not provide adequate control for more than a few years if the area is left as grassland.

It is suggested that the observed increase in wireworm numbers after initial control, while a true resurgence, was a type that is probably more prevalent in arable land than is generally realized. The phenomenon is not attributed to the reduction of predators or competing arthropods, although these factors probably had an unmeasured influence, but chiefly to the improvement of the floral environment which was of benefit to the principal pest species after the insecticide had dissipated.

As a corollary, the study implies that since certain soil inhabiting insects are apparently capable of causing considerable floristic changes, they should not be neglected as ecological factors in grassland succession.

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<sup>8</sup> Scientific nomenclature follows Gray's "Manual of Botany", 8th ed., by M. L. Fernald.

# Seed Dressings for Control of Maggots and Diseases Attacking Certain Field and Vegetable Crops in Southern Ontario, 1956<sup>1</sup>

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## ABSTRACT

Control of the seed-corn maggot, *Hylemya cilicrura* (Rond.), the most serious soil pest of peas, corn, beans, and cucurbits in southern Ontario, was obtained by coating 100 lb. of seed with 0.4 oz. of heptachlor, dieldrin, or lindane combined with 1.5 oz. of thiram or captan. A seed dressing containing an insecticide or fungicide alone was in some cases ineffective. On Yellow Globe cooking onions, smut, caused by the fungus *Uroceptis cepulae*, and the onion maggot, *Hylemya antiqua* (Meig.), were effectively controlled with a seed dressing containing 2 oz. of thiram and 1 oz. of heptachlor or dieldrin per pound of seed.

The amounts of protectants remaining on the seed coats following germination of certain seeds are discussed. It is suggested that the planting rates for many crops be reviewed in the light of increased crop protection afforded by effective seed dressings.

## INTRODUCTION

The use of seed dressings for the combined control of insects and disease is one of the most important recent developments in crop protection. Combinations of insecticides and fungicides at varying rates and under different soil conditions for the control of the seed-corn maggot, *Hylemya cilicrura* (Rond.), and a number of soil pathogens have been studied in southern Ontario since 1951. The project was initiated because of the interest of the canning companies in the processing of lima beans. The canning companies schedule the seeding of the lima bean crop over a two- to three-week period to obtain a continuous flow of produce at harvest. In some years large acreages had to be re-seeded because of severe maggot infestations and subsequent seed decay. Re-seeded fields matured later and disrupted the harvest schedule so that quality and yields were frequently adversely affected.

The seed dressing trials, which began with lima beans, have been expanded and now include practically every crop known to be susceptible to the seed-corn maggot in southern Ontario. Although the data reported here were obtained in 1956, the conclusions are based on four years' experimental evidence.

## MATERIALS AND METHODS

Table I shows the insecticidal and fungicidal formulations tested. The seed protectant was applied in slurry form to beans, corn, cucurbits, and peas using a 4 per cent methyl cellulose solution as the liquid carrier and as a sticker. The rate of application was 0.4 oz. of actual insecticide and 2.0 oz. of fungicide formulation per 100 lb. of seed.

Four replicates of 50 seeds per replicate were hand-sown for each crop. Stands were recorded three weeks after seeding.

## RESULTS AND DISCUSSION

### BEANS AND CORN

Previous experiments at Chatham have shown that under optimum growing conditions and in the absence of maggot infestations, little is gained from seed dressings providing high quality seed is used. However, under adverse growing conditions germination is retarded, and seed treated with a fungicide alone is afforded considerable protection from soil pathogens.

The results of seed dressing trials on beans and corn in 1956 are given in Table I.

Infestations of the seed-corn maggot are invariably more severe in backward springs (Miller 1954) such as that experienced in 1956 in southern Ontario. Seed treated with an insecticide alone is generally partially protected from soil pests. However, at other times,

<sup>1</sup> Contribution No. 3597, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

TABLE I—Stand Obtained with Various Crops Three Weeks after Seeding, Chatham, Ontario, 1956.

Seed Dressing	Number of plants per 200 seeds						
	Field beans	Green pod	Lima	Kidney	Soybeans	Corn	Total
Heptachlor <sup>1</sup> + Arasan <sup>2</sup>	191	157	163	176	175	186	1048
Dieldrin <sup>3</sup> + Orthocide <sup>4</sup>	181	156	169	179	173	190	1048
Dieldrin <sup>3</sup> + Arasan <sup>2</sup>	187	158	159	170	177	188	1039
Heptachlor <sup>1</sup> + Orthocide <sup>4</sup>	176	161	159	180	177	182	1035
Ortho Seed Guard <sup>5</sup>	189	154	156	172	181	181	1033
Heptachlor <sup>1</sup> + Thioneb <sup>6</sup>	183	153	156	178	167	187	1024
Dieldrin <sup>3</sup> + Thioneb <sup>6</sup>	189	154	155	168	171	185	1022
Heptachlor <sup>1</sup> + Spergon <sup>7</sup>	182	147	141	170	180	187	1007
Dieldrin <sup>3</sup> + Spergon <sup>7</sup>	168	143	143	160	167	193	974
Heptachlor <sup>1</sup>	190	152	118	169	161	177	967
Orthocide <sup>4</sup>	150	140	150	149	161	184	934
Spergon <sup>7</sup>	162	134	136	144	161	184	921
Arasan <sup>2</sup>	146	143	131	155	148	188	911
Thioneb <sup>6</sup>	153	134	147	126	164	183	907
Dieldrin <sup>3</sup>	180	125	95	159	155	169	883
Check	149	117	104	123	139	160	792
L.S.D. @ 1 per cent level	7.0	6.9	7.1	7.1	7.2	5.1	51.1

<sup>1</sup> Velsicol Chemical Corporation, Chicago, Ill.; 75% w.p.  
<sup>2</sup> Canadian Industries Limited, Montreal, P.Q.; 75% thiram.  
<sup>3</sup> Shell Chemical Company of Canada, Toronto, Ont; 50% w.p.  
<sup>4</sup> California Spray-Chemical Corporation, Richmond, Cal.; 75% captan.  
<sup>5</sup> California Spray-Chemical Corporation, Richmond, Cal.; 16.5% lindane plus 50% captan.  
<sup>6</sup> Naugatuck Chemicals, Elmira, Ont.; 50% polyethylene thiram sulfide.  
<sup>7</sup> Naugatuck Chemicals.; Elmira, Ont.; 95% chloranil.

seed treated only with an insecticide appears to be predisposed to attack from soil pathogens, and plant stands are actually less than in the untreated checks. This peculiar relationship is illustrated by the lima beans treated with dieldrin alone, Table I, and the watermelon and peas treated with dieldrin or heptachlor alone, Table II. Adverse effects from seed dressings containing only an insecticide have been observed by Howe and Schroeder (1951) and Natti and Schroeder (1955) in New York, and Hofmaster and Nugent (1956) in Virginia. It is conceivable that a naturally-occurring protective microflora on the seed coat, such as that found by Chinn (1954) on wheat, is rendered partially ineffective by the addition of an insecticide.

In general, those combinations of dieldrin or heptachlor with Arasan or Orthocide were somewhat superior to those containing Spergon or Thioneb as the fungicides. The lindane-Orthocide combination was also very effective. When maggots are present, maximum effectiveness of the protectant is obtained only when the insecticide and fungicide are in combination. Maggot-damaged seeds are susceptible to *Fusarium*, *Phytophthora*, *Rhizoctonia*, and *Pythium* organisms, and the addition of an effective fungicide to the seed dressing prevents or retards the invasion of these fungi. Heptachlor was consistently better than dieldrin when not in combination with a fungicide.

In 1952, the effectiveness of a seed treatment was successfully demonstrated on lima beans, and since then all lima beans grown in southern Ontario on a contracted acreage basis have been treated with a combination insecticide-fungicide as an insurance against insect and disease damage. No re-seeding has been necessary and the canning companies have been able to schedule planting dates with a degree of confidence not possible prior to the use of seed dressings.

A further advantage of this method of insect and disease control is that seed can be treated during the winter. The results of experiments at Chatham in 1954 and 1955 showed that pea, corn, soybean, kidney bean, lima bean, snap bean, and field bean seed could be



TABLE II—Stand Obtained with Various Crops Three Weeks after Seeding, Chatham, Ontario, 1956.

Seed Dressing	Number of plants per 200 seeds						
	Cucumber	Musk-melon	Pumpkin	Squash	Water-melon	Peas	Total
Dieldrin + Arasan	149	150	188	138	169	131	925
Dieldrin + Orthocide	161	153	175	144	161	125	919
Heptachlor + Orthocide	161	134	191	156	162	114	918
Dieldrin + Thioneb	168	133	183	154	145	114	897
Heptachlor + Thioneb	152	136	187	144	154	109	882
Ortho Seed Guard	171	143	180	181	164	40	879
Heptachlor + Arasan	156	130	176	140	152	119	873
Orthocide	149	107	182	150	159	117	864
Arasan	148	107	185	123	144	120	827
Heptachlor + Spergon	142	114	180	121	126	118	801
Thioneb	137	114	193	131	127	80	782
Dieldrin + Spergon	144	113	172	105	122	95	751
Heptachlor	159	114	184	133	84	58	732
Spergon	146	89	180	117	112	80	724
Dieldrin	146	104	182	109	77	50	668
Check	135	100	171	100	91	62	659
L.S.D. @ 1 per cent level	7.5	12.9	n.s.	6.9	7.4	8.9	39.1

treated at least five months prior to planting without adverse effects to germination and yield.

#### CUCURBITS AND PEAS

The 1956 trials with cucurbits gave promising results. In previous years, reports of infestations of the seed-corn maggot in the seeds of cucurbits have been rare although probably more widespread than believed. Consequently, the need for protection during germination was not as apparent as on beans. With the exception of Connecticut Field Pumpkin, all combined dressings significantly increased germination over the checks. From these initial trials, it would appear that Connecticut Field Pumpkin may be less susceptible to maggot attack and soil pathogens than other cucurbits. Maggots were present in all the plots with the exception of pumpkin.

The seed-corn maggot has seriously damaged laboratory plots of canning peas for the past three years. Although field infestations of economic importance have not been observed in southern Ontario, there is a possibility that the insect will become a major pest of this crop. Peas are planted in the early spring when the overwintering generation of the seed-corn maggot is emerging and, theoretically at least, should be an ideal host for this generation. When planted at the rate of 4 to 6 bu. per acre, yields of shelled peas have averaged about 2500 lb. per acre over the past five years and, according to many agriculturists, this yield is approximately 16 per cent of a theoretical maximum.

In repeated trials in a greenhouse at Chatham using untreated seed grown in garden soil, germination was extremely poor. By growing untreated seed in sterile soil, germination reached almost 100 per cent; similar results were obtained by growing treated seed in soil contaminated with decay organisms. Thus it would appear that canning peas are extremely susceptible to seed decay, and that a seed dressing containing at least a fungicide should be recommended. The addition of an insecticide would add little to the cost and give protection against possible infestations of the seed-corn maggot.

The germination of good canning pea seed should average about 94 per cent. Since only a 65.5 per cent plant stand was obtained with the best seed dressing, dieldrin plus Arasan, Table II, it is evident that more field trials are required before the maximum germination is approached.

The effectiveness of the various combinations on cucurbits and peas is comparable to that on beans and corn. Those combinations containing Arasan or Orthocide were generally more effective than those containing Thioneb or Spergon. The unaccountably poor results

obtained with the commercial lindane-Orthocide preparation are not consistent with previous field and greenhouse trials when the material compared favorably with others tested.

ONIONS

In the muck soils of southern Ontario smut, caused by the fungus *Uroceptis cepulae*, occurs annually and would limit onion production were an adequate control not available. A 1 per cent formaldehyde solution directed into the trench during seeding is effective, but is not popular in areas where water is scarce. Smut is a minor problem in the mineral soils, and preventative measures are generally not adopted. The onion maggot, *Hylemya antiqua* (Meig.), occurs annually in the muck and mineral soils, but the severity of infestations varies from year to year.

In an experiment in 1956, using several fungicides in combination with heptachlor, Arasan was superior to Thioneb, Orthocide, or Spergon for the control of onion smut, Table III. All combinations gave outstanding control of the onion maggot. In similar experiments in 1955, dieldrin was equally effective to heptachlor.

TABLE III—Smut and Onion Maggot Control in Yellow Globe Onions, Chatham, Ontario, 1956\*.

Material	Rate (oz. per lb.) active ingredient	Per cent smut	Per cent maggot
Arasan	2	0	0
"	4	3	0
"	8	0	0
Thioneb	2	14	< 1
Orthocide	2	14	0
Spergon	2	16	< 1
"	4	19	0
Check	No treatment	38	27

\*Heptachlor 75 per cent W.P. @ 1 oz. actual per pound was used in all treatments.

Where maggots and not smut are a problem in Ontario, the use of insecticidal seed dressings will undoubtedly increase. Seed dressings are much cheaper than the present broadcast soil treatment for maggot control. In areas where maggots and smut occur together, excellent control has been obtained by adding an insecticide in emulsifiable form to the formaldehyde solution. This is a simple, comparatively inexpensive method and is increasing in popularity even in areas where water is scarce.

RETENTION OF PROTECTANT ON SEED COATS

An interesting problem associated with seed dressings is the amount of protectant adhering to the seed coat following germination. During germination the cotyledons and seed coats of many seeds are forced above ground. The number of seed coats forced above ground depends in large part, on the type of soil; for example more seed coats are obtained from onions in loose, friable muck than in heavier soil.

TABLE IV—Retention of Toxicant on Seed Coats of Yellow Globe Onions, Chatham, Ontario.

Treatment	Method	Rate (oz. actual per lb.)		Per cent retained	
		Fungicide	Heptachlor	Fungicide	Heptachlor
Spergon SL	Dry	4	—	89	—
Arasan SFX	Dry	4	1	74	51
Arasan SFX	Pelleted*	4	1	119	74

\*Slurry treated with 4 per cent methyl cellulose sticker.

The data in Table IV were obtained from numerous trials in which the seed coats were collected after they had been forced above ground and analyzed to determine the amounts of protectants still present.

The retention of 119 per cent of Arasan shown in column five resulted from an inadequate sample. It is rather surprising that such a large proportion of the protectants is carried above ground and is, therefore, ineffective. It is thus conceivable that a seed dressing might fail under certain conditions.

In 1956 preliminary trials were conducted to determine the effect of soil moisture content on the retention of protectants on the seed coats. Seed sown in soil with only sufficient moisture for germination was compared with that in soil kept continually saturated. Table V shows that most of the protectant remained in the wet soil following seed germination, and less than one-half remained in the slightly moist soil. Thus, seed dressings should be comparatively more effective in wet seasons because more protectant remains in the soil where it is required.

TABLE V—Retention of Thiram on Bean Seed Coats Treated\* with Arasan SFX at 2 oz. per Bushel, Chatham, Ontario, 1956.

Seed	Per cent thiram retained on seed coats	
	Slightly moist soil	Wet soil
Lima bean	58	3
Field bean	73	13
Soybean	39	2
Red kidney bean	56	20

\*4 per cent methyl cellulose was used as a sticker.

## CORRELATING PLANTING RATES WITH SEED PROTECTION

The planting rates for various crops should be reviewed in the light of results obtained with effective seed dressings. Previous to the use of seed dressings, planting rates were established on the assumption that losses attributable to insects and disease were inevitable. Now, when a high degree of control can be reasonably expected, it is possible to increase the plant stand without obtaining a comparable increase in yield and, occasionally, to the detriment of the yield. At Chatham in 1954, seed dressing trials on soybeans resulted in an increased stand of 26 per cent, but an increase in yield of only 7 per cent. In the same year seed dressing trials on onions, in which practically 100 per cent control of the onion maggot was obtained, resulted in decreased quality and yield of the onion crop. By increasing the number of seedlings per unit of area, crowded conditions developed and at harvest the proportion of No. 2 to No. 1 grade onions, based on diameter, was far too high. The check plots returned a significantly higher yield of No. 1 grade onions in spite of the initial losses caused by a moderate maggot infestation.

Testing fungicidal seed dressings on selected, healthy soybean seed in southern Ontario, Hildebrand and Koch (1947) were able to demonstrate that although plant emergence was closely related to planting rates of 20, 30, 40, and 50 lb. per acre, significant differences in yields were not obtained. Soil insects were apparently not a factor in these trials.

Results such as these indicate that where increased stands are obtained with seed dressings, a downward revision of planting rates should be possible and desirable. For every crop there is an optimum planting rate for optimum yield under optimum growing conditions. The use of effective seed dressings brings optimum growing conditions closer to reality.

## ACKNOWLEDGEMENTS

The authors thank J. R. W. Miles and W. W. Sans of the Chemistry Division, Science Service, Chatham Laboratory, for determining the residues on the seed coats, and A. J. De Lyzer of the Chatham Laboratory for assistance in the field.

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# Ten Years' Field Study of Methods of Evaluating Root Maggot Damage and Its Control by Chemicals in Early Cabbage<sup>1</sup>

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## ABSTRACT

During the 10 years, 1946–1955, that the bionomics and control of the cabbage maggot, *Hylemya brassicae* (Bouché), were studied at Victoria, British Columbia, eight large-scale field experiments were conducted with early cabbage to test 41 treatments using 10 insecticides and five methods of application. This paper presents representative data from the study of five methods of field evaluation of the treatments. For such evaluation it was necessary to measure accurately the damage done by the maggots and its reduction by treatment, especially for the critical period between transplanting and establishment of the cabbage plant.

Yield on an area basis provided the best summation of the over-all effects of attack and of the direct and indirect effects of the chemical treatment in relation to environmental factors. Yields from different experiments were best compared when expressed as a percentage of the yield of the highest-yielding treatment of its own experiment. Determining numbers of plants ruined by maggots provided by far the best single method of treatment evaluation under the conditions at Victoria. Damage rating was useful but was laborious, subjective, and not always possible. Counts at harvest time of plants infested and of larvae and pupae on the roots were the least useful of the methods employed. Whatever the criteria, only years of moderate to severe infestation provide critical tests of treatment effectiveness.

From 1946 through 1955 the cabbage maggot, *Hylemya brassicae* (Bouché) was studied at Victoria, British Columbia. During eight of those years, i.e., excepting 1946 and 1953, large-scale field experiments on chemical control were conducted in early cabbage (King, Forbes, and Noble, 1957). The manner in which root maggots attack the host plant was studied to develop and compare methods of appraising the damage caused and the effects of chemical controls. Seven methods of evaluation were tried, five being employed in most of the experiments.

## SPECIES AND LIFE HISTORY

As a pest the cabbage maggot, *Hylemya brassicae* (Bouché), was the primary species involved in all the experiments. The secondary *H. cilicrura* (Rond.)—*H. trichodactyla* (Rond.) complex was sometimes found in much smaller numbers on the roots in association with the cabbage maggot, especially late in the season. The maggots, *Muscina assimilis* (Fall.) and *Fannia canicularis* (L.), were often abundant on roots affected by clubroot.

Infestation by the first-generation of the cabbage maggot is the critical factor in the production of early cabbage in coastal British Columbia. Flies begin to emerge from overwintering puparia during the first long warm period in April or May and about a week thereafter to lay eggs. Over a nine-year period on the farm on which the present experiments were conducted, egg-laying most commonly began late in April, the earliest and latest dates being April 23 and May 31, respectively.

At Victoria, early cabbages are transplanted to the field during late April or early May. Consequently, they receive the heavy spring egg-lay while they are still small and not fully established. Peak deposition of first-generation eggs is usually reached by late May. Early cabbage crops also receive considerable numbers of second-generation eggs but since the plants are nearly mature by this time, second-generation infestation does not materially affect production.

Infestations in the experimental plots varied from very light to very severe with from one per cent, 1947, to 90 per cent, 1955, of the untreated plants being ruined by cabbage maggot attack.

<sup>1</sup> Contribution No. 3621, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

## EXPERIMENTAL TECHNIQUE

All experiments were adequately replicated with plots randomized in the standard way. Randomized block design was used each year except 1950 and 1951, when latin square design was utilized. There were generally 40 to 60 plants per plot.

Plants were started in the greenhouse or in seed beds protected from infestation. They were transplanted into the field generally in late April or early May.

Periodic inspections of all plants were made throughout the growing season to locate dying plants and determine cause of death, and to record other pertinent data such as indications of phytotoxicity. Individual plant records were maintained. Harvest data were taken as the groups of plants matured. Statistical evaluation of results was by analysis of variance. Plants killed or made non-productive by cultivation injury, flooding, wirestem and clubroot diseases, attack by muskrats and cutworms and similar causes were not included in the compilation of final results. The number of plants thus involved was usually very small.

## MATERIALS AND APPLICATION

Ten insecticides were used in 41 treatments, and five methods of application. Varieties of cabbage used included Green Acre, Golden Acre, and Cluseed.

## ASSESSMENT OF DAMAGE AND CONTROL

Several quantitative criteria were used for evaluating the effectiveness of insecticidal treatments. The five used for ranking were: number of plants ruined by maggots, number of plants infested, numerical damage rating, number of larvae and puparia about the roots at harvest, and yield.

PLANTS RUINED were those killed during the growing period by root maggots and those that, because of root maggot attack, failed to produce firm marketable heads weighing 12 ounces or more by the last harvest. For analysis, the number of plants ruined was expressed as a percentage of the total plants.

PLANTS INFESTED were determined at harvest from a predetermined number of head-bearing plants, usually 20 or 25, taken systematically from the central section of each plot. Each plant was classified as infested or not infested. For analysis, the number of plants infested was expressed as a percentage of the plants producing marketable heads.

DAMAGE RATINGS were determined at harvest when the same plants that were used to determine infestation were examined for appraisal of the amount and seriousness of root maggot damage. The roots were dissected to determine depth of penetration of feeding and whether maggots had bored up the main tap root early in the season. The damage was then assessed as light, moderate, or severe and assigned factors of 1, 2, and 4. A clean root rated 0. From these data, a figure representing the damage rating per head-producing plant was calculated for each plot.

NUMBER OF LARVAE AND PUPARIA was determined at harvest from the same group of plants as above. A two-minute examination was made of the roots and soil about each plant to determine the larvae and puparia present. Data for each plot were expressed as number per head-producing plant.

YIELDS were based on the marketable heads cut at each harvest from each plot. The total weight and number of heads involved were recorded, the average weight per head being calculated from these data. Yields per acre of 10,000 plants were calculated from weight per head and per cent ruined. Thus, if 10 per cent of the plants in a treatment were ruined and the average weight of the heads produced was 40 ounces, the approximate yield per acre was 11.25 tons.

OTHER METHODS included, in 1947–1952, weighing each head. Measuring the diameter of each head was also tried in 1948 but was not satisfactory.

## RESULTS

Root maggots injured the cabbage in several ways. In young plants stem boring was characteristic. The newly hatched larvae entered the tender underground stem, leaving

little external sign of their entry, and bored up and down within the pith. This type of injury was nearly always fatal, even when only one larva was present. Attack at the growing point of the main root was also frequently fatal to young plants and caused so much injury even to older, better established, plants that they seldom produced marketable heads. In larger, succulent plants maggot attack was almost entirely external. Heavy infestation was sometimes fatal but more typically a plant so affected survived to produce a small head, usually of poor quality. Such plants had a characteristic dull gray-green appearance of the leaves with a tendency to wilt on hot days. Maggot attack on a nearly mature plant had little apparent effect upon the size or quality of head produced.

Table I presents detailed data from the 1952 experiment for all five methods of evaluation, two columns based on yield data being given. These results are representative of those for other years.

TABLE I. Comparison of Six Criteria for Evaluating the Effectiveness of Chemical Treatments Against the Cabbage Maggot, *Hylemya brassicae* (Bouché), on Early Cabbage at Victoria, B.C., 1952.

Treatment	Harvested plants				Total plants	
	Infested, per cent	Damage rating per plant	Number of larvae and puparia per plant	Average weight per head, ounces	Ruined, per cent	Yield per acre, tons
Aldrin spray	10	0.1	0.1	34	0	10.9
Aldrin dip	15	0.2	0.3	29	0.6	8.9
Heptachlor dust	49	0.7	1.8	33	0.6	10.2
Aldrin dust	80	1.3	3.7	26	1.3	8.5
DDT dip	84	1.4	2.3	33	2.2	10.0
BHC dust	96	1.9	4.9	29	6.8	8.7
Chlordane dust	93	1.6	6.0	28	11.5	8.0
Check	99	2.7	7.9	26	33.6	6.0
LSD at 5% level	15	0.4	2.0	6	8.7	1.6

Table II gives certain correlations between the various appraisal methods for several of the experiments.

TABLE II. Correlation of Yield per Acre and Weight per Head with Other Evaluation Criteria for Several Experiments, 1948-1955.

Correlation	1948	1949	1950	1952	1953	1955	Average
Yield per acre and							
% ruined	-.90	-.93	-.87	-.89	-.84	-.99	-.90
pupal counts	—	-.85	-.82	-.87	-.78	—	-.83
damage rating	—	-.92	-.69	-.80	-.83	—	-.81
% infested	-.86	-.89	-.71	-.63	-.62	—	-.74
Weight per head and							
% ruined	-.67	-.73	-.67	-.58	-.65	-.98	-.71
pupal counts	—	-.87	-.60	-.71	-.60	—	-.69
damage rating	—	-.94	-.53	-.62	-.65	—	-.68
% infested	-.87	-.86	-.47	-.54	-.28	—	-.60
% ruined in check	41	48	23	34	23	90	43

### GENERAL DISCUSSION

It seems significant that yield per acre shows higher correlations than does weight per marketable head, not only with per cent ruined, but also with pupal counts, damage rating, and percentage infested (Table II). This holds true even though the three latter criteria are derived from exactly the same groups of plants as is weight per head, whereas yield and per cent ruined apply to total plants. In all instances, the correlations between measurements of yield and measurements of maggot effects tend to be higher in seasons of heavy infestation, while in years of moderate infestation the correlations are lower and more erratic (Table II).

Damage by root maggots to cabbage and other stem brassicas is measured mainly by plant survival and to a lesser degree by the yield of the surviving plants. This is almost completely opposite to the criteria used for rutabagas and other root crucifers, in which damage is measured by quality and marketability rather than gross yield (King & Forbes 1954, King *et al.* 1955, Forbes & King 1956). The methods of appraisal for the two problems are therefore very different.

For cabbage, accurate diagnosis of damage sufficiently severe to kill or ruin the plant was nearly always possible when the plants were examined as soon as they were clearly beyond recovery. For plants surviving to harvest, careful examination permitted reliable diagnosis provided there were no serious complicating factors such as very extensive clubroot.

A method of appraisal must be statistically sound and must measure accurately and quantitatively the damage caused by root maggots and its modification by chemical control in terms that have practical significance for growers. The data should be obtainable with a minimum expenditure of time. Partial criteria that measure one or more aspects of the relationship between infestation and resultant injury are, however, useful research tools.

To the growers, it is the yield per acre of marketable cabbage that mainly determines the acceptability of a control treatment. Yield data take into account:—(1) loss of plants ruined by maggot, including those killed at an early stage and those that survive but fail to produce marketable heads; (2) loss of weight in the marketable heads produced by infested plants; and (3), gains or losses from the stimulating or phytotoxic effects of the chemical on plant growth and the indirect effects of control of other insects. Thus only yield, on an *area* basis provides the essential summation of the over-all effects of a treatment. Moreover, yield data on a plot basis are obtainable relatively easily and quickly and are relatively consistent.

A weakness of yield data arose from the high degree of biological variability in cabbage emphasized by environmental factors. In light infestations significant differences between treatments in infestation and damage were not always reflected in significant differences in yield. In spite of these weaknesses, yield data gave the best over-all evaluation and were the base from which all other criteria of root maggot infestation and damage were best measured (Table II).

For comparisons, it was found best to express the yields in relative rather than absolute terms. In each experiment the yield for the best yielding treatment was taken as a standard and the yields for all treatments were expressed as percentages of it. The standard was a measure of the potential yield for the season, variety, and field. This procedure eliminated as far as possible the factor of root maggot infestation since the best-yielding treatment was always one in which the greatest degree of control of root maggots had been obtained. Along similar lines Wright (1953) suggested that damage caused by root maggots on brassica crops be assessed by recording the yield from two series of plots, in one of which the pest had been controlled.

The number of plants ruined was rapid and simple to determine. Those plants dying during the growing season were immediately pulled and examined to determine cause of death. At harvest only those non-productive plants that were still living had to be examined. In both groups results were usually clear-cut. Damage represented by the percentage of plants ruined was directly reflected in the yields since, under British Columbia conditions, replanting is not practical commercially even for plants killed soon after transplanting.

The percentage of plants infested has long been used as a criterion of root maggot damage. The present studies, however, have shown that under British Columbia conditions it only partially represents the critical factors of infestation, i.e., when and where it occurred and to what extent it caused actual damage. Moreover, it requires substantially more time to determine plants infested than plants ruined. For the former, each root must be examined at harvest, often very painstakingly. Occasionally, it is not even possible to secure the data with accuracy, e.g., in the 1955 experiment in which severe clubroot masked evidence of feeding by root maggots and induced infestation by maggots of secondary species.

Rating of the damage caused by root maggots was used from the beginning in the present experiments because of its research value. On theoretical grounds it is evident that



when the feeding by maggots can be accurately assessed at harvest time by visual examination of the roots of head-bearing plants, and combined with a suitable rating for the ruined plants, the resulting index will provide a close measure of the total damage by root maggots. The data in Table I are partial indices limited to the head-producing plants. They, nevertheless, show good correlation with yield, and fair with weight per head (Table II). The method of rating damage is necessarily subjective and time consuming, and its usefulness is lessened when the second-generation maggots become abundant on the plants just before harvest. Moreover, maggot damage cannot be appraised by direct examination where clubroot growth is extensive.

Larval and pupal counts measure the insect population present at harvest and thus provide useful biological data. In some instances, such counts fairly represent the maggots responsible for the most critical feeding, and bear a close relation to damage and yield. Typically, however, by harvest time it is difficult to locate the empty puparia of the larvae responsible for the critical early damage. In such instance, the counts may bear little relationship to the severity of damage. These counts are time-consuming, even where restricted to two minutes per plant (Eide & Stitt 1950); and where secondary species are present in numbers much further time is required for identification.

Statistically, each of the five methods was found to provide a consistent means of differentiating treatments except that under conditions of light infestation, yield data sometimes failed to give significant differences. Data on weight per head were not stable; but yield on an area basis was considerably more reliable, being stabilized by inclusion of plants-ruined data in its calculation. Furthermore, in any one year, the relative ranking of treatments was, apart from minor changes due to statistically non-significant differences, the same for each of the methods, except occasionally those based on yield as noted above (Table I).

As shown in Table II, of all of the methods of evaluation per cent ruined showed the highest correlation with yield. Good correlations with yield were shown by pupal counts and damage ratings. The correlation between per cent infestation and yield was erratic.

Each of the methods of appraisal gave worthwhile information on the performance of the various insecticidal treatments. From the standpoint of practical control, plants ruined and yield together provided the most significant and easily obtainable methods of overall evaluation. This conclusion agrees with that of Brittain (1920) whose findings unfortunately have been neglected in much of the work since his time.

In these experiments, only the years when one-quarter or more of the untreated plants were killed by maggot attack afforded reliable tests of treatment effectiveness. In those years, counts of plants ruined by maggots provided in themselves an easy and satisfactory rating of treatments.

### ACKNOWLEDGMENTS

Mr. M. D. Noble, Technician, gave extensive and invaluable assistance throughout the greater part of these investigations. The authors gratefully acknowledge the assistance of A. T. S. Wilkinson, Associate Entomologist, in taking harvest data on several occasions.

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# Soil Insect Pests of Corn and Their Control in North Carolina<sup>1</sup>

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## ABSTRACT

The most important soil insect pests of corn are various species of wireworms of the genera *Melanotus*, *Conoderus*, and *Glyphonyx*. Billbugs (*Calendra* spp.), cutworms (chiefly *Agrotis ypsilon* (Rott.)) and other soil pests are sometimes very destructive. In many fields, more than one pest may be damaging corn. Soil treatment with aldrin, dieldrin or heptachlor at 2 pounds per acre has resulted in good control.

## INTRODUCTION

The increasing importance of corn in the agricultural economy of North Carolina is becoming ever more apparent. Heretofore, the production and storage of grain has been regarded as a local farm affair. However, the introduction of new corn varieties, better agricultural practices and closer attention to the economics of production has boosted production, and, at the same time, has stimulated the grower's need for a larger market for his grain. This, in turn, has made the grower more aware of the necessity for controlling insect pests of corn in order to reduce production costs. The high cost of hybrid seed (up to \$10.00 a bushel) makes the farmer realize that he cannot afford to lose as much of it as he can of the less expensive open-pollinated varieties of corn.

In the past, many growers have been content to accept failures in corn stands as inevitable. Usually the reason for failure was ascribed to poor seed, poor seed-bed preparation, land being too "stiff" or too "cold", bird damage, and similar factors. However, when growers began to use expensive hybrid seed, they became more anxious to determine definitely the reasons for stand failure. Now it is rare to find a corn grower who is not aware of the importance of the correlation between soil insects and stand failures.

In North Carolina, we have a variety of subterranean insect pests. Most of our soil pests attacking corn are Coleoptera, but Lepidoptera are sometimes very damaging. Corn is subject to insect damage from the time the seed is placed in the ground until the time of harvest. Usually, most of the damage by soil pests occurs during the first two months of the plant's growth. The seed, the roots, and the underground portion of the stem may be attacked and fed upon by a variety of subterranean pests.

## SEED PESTS

As soon as the pericarp of the corn kernel begins to soften, damage by several pests may begin. Our principal seed pests are various species of wireworms (Elateridae: *Melanotus communis* (Gyll.), *Conoderus lividus* (DeG.), and *Glyphonyx* spp.). Penetration of the kernel at one vital spot by any of these larvae may be sufficient to stop germination of the seed. In severe cases of infestation, I have seen as many as 20 to 28 *M. communis* larvae in the vicinity of one seed. In such a heavy infestation, the seed is completely eaten and usually all that remains of the seed is a shred or two of the pericarp.

Other seed-attacking pests are the seed corn maggot (Anthomyiidae: *Hylemya cilicrura* (Rond.)) and the southern corn rootworm (Chrysomelidae: *Diabrotica undecimpunctata howardi* Barber). The former is usually found in dark soils where much green vegetation has been plowed under just before planting. The southern corn rootworm is very common throughout the entire state in low, poorly drained areas of the field. Ordinarily, the rootworm is more damaging to the underground portion of the stem than it is to the seed.

## ROOT PESTS

The species of wireworms which attack the seed also feed on the roots, which may be eaten entirely or tunneled into. In the case of root feeding by *Melanotus communis* or *Conoderus lividus* the damage may be very severe, resulting in stunting of plant growth and usually progressing to the death of the plant. Injury by *Glyphonyx* larvae is not as severe

<sup>1</sup>Contribution from the Entomology Department, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research as Paper No. 753 of the Journal Series.

although plant death may occur because of root feeding by these larvae. An unusual root pest is the sand wireworm, *Horistonotus uhlerii* Horn. Its shape, color, and movements are very much unlike the other species described before. It is white in color with a brown head capsule. It moves in a disjointed manner. Its distribution in North Carolina is limited to the southern part of the state in the very sandy soils of Columbus County and to some extent in similar soils in a few adjoining counties. This species feeds on the small roots of the plant causing it to be stunted and slow-growing. Killing of plants is not as pronounced as in the case of the other mentioned wireworms, but unthrifty stunted plants do not produce ears.

Root damage may also be caused by mole crickets (Gryllotalpidae: *Gryllotalpa hexadactyla* Perty), white grubs (Scarabaeidae: *Phyllophaga* spp.) and the grape colaspis (Chrysomelidae: *Colaspis flava* (Say)).

### STEM PESTS

*Melanotus communis* and *Conoderus lividus* may also be serious stem pests. As the plant grows older, more soil is thrown around the base of the stem in the usual process of cultivation. Frequently, wireworms will be found in the stem just above the root crown or slightly higher. They bore into the stem, thus in effect girdling it and the plant dies soon after. These wireworms ordinarily do not tunnel up and down the stem.

Two species of billbugs (Curculionidae) are serious pests of young corn throughout eastern North Carolina. *Calendra callosa* (Oliv.), the clew bug, is generally well distributed throughout eastern North Carolina. *Calendra maidis* (Chitt.), the maize billbug, is found in small areas in southeastern and eastern North Carolina. Serious damage to young corn seedlings results from adults feeding on the stem. A characteristic sign of billbug damage is a row of four elongate holes transversing a blade of corn. Later on, adult billbugs feed on the stem just at the soil level. Young plants may be completely destroyed and older plants are stunted and never produce a full ear.

In cool, wet springs, the black cutworm (Phalaenidae: *Agrotis ypsilon* (Rott.)) may be a serious pest. Plants up to six inches may be cut off level with the ground. Damage seems to be more severe in soils that are "cloddy", a condition usually caused by cultivating the ground when it is too wet.

Frequently, the rough-headed corn stalk borer (Scarabaeidae: *Euetheola rugiceps* Lec.) attacks corn planted after sod in poorly drained areas. The adults chew on the base of the stem often eating half way through the stem. Damaged plants usually do not recover.

Another stem feeder, not of frequent occurrence, is a webworm (Crambidae: *Crambus* sp.). Young larvae bore fine holes in the tips of the leaves of young corn and then proceed down the stem making a web at the side of the stem just below the soil surface. The stem is chewed at this point causing the plant to become stunted and unthrifty in growth.

These are the more common of our subterranean species. Some years, other species may occur. In our 1955 test plots on one farm in eastern North Carolina, we recorded 17 different species of subterranean insects doing damage to corn.

### METHODS OF CONTROL

The need for quick control methods has given impetus to chemical control measures. There are three basic methods of chemical control: (1) treating the seed; (2) treating the soil; and (3) mixing the insecticide with the fertilizer and applying at the time of planting. In some early tests against wireworms we have tried these three basic methods of chemical treatment as well as combinations of them. Work previously reported (Kulash and Monroe 1955) showed that the poorest control was obtained with seed coating, which did not give adequate protection against wireworm attack. A survey of growers' use of this method (Kulash, 1954) also showed that there were many failures when seed coating alone was used to control wireworms.

We have also had to be cautious about using mixtures of insecticides with fertilizer applied in bands to control wireworms attacking corn. Although this method has given good control with some wireworms and other subterranean pests, we have had failures where there have been heavy populations of *Melanotus communis*. The reason for this is quite simple: as the plant grows, soil is thrown around the stem in the usual process of cultivation, and wireworms are able to avoid the insecticide-fertilizer bands by attacking the stem where this fresh dirt has been placed around it.



Because of the inadequacy of seed-treatment and insecticide-fertilizer mixtures for wireworm control, we have stressed soil treatment as the best chemical control method. In this method, commonly referred to as the "broadcast" method, insecticidal dust or spray is applied directly to the soil surface. Land should be prepared as for planting before the application is made. After application the land is disked and cross-disked to a depth of three or four inches in order to mix the insecticide with the soil.

We have tried the broadcast method of application in our tests against wireworms (*Melanotus communis*, *Conoderus lividus*, and *Glyphonyx* spp.), billbugs (*Calendra callosa*, *C. maidis*) and the black cutworm (*Agrotis ypsilon*). Usually, other subterranean pests have been present in our experimental plots. Our results have been favorable (see Tables I, II, and III). Corn planted in plots treated one year previously with two pounds per acre of actual dieldrin showed less billbug damage than did untreated check plots (see Table IV).

TABLE I—Summary of Wireworm Control in Soil Treatment Tests, Hyde County, North Carolina—1955.

Treatment (2 lbs./A of Active Ingredient)*	Total No. Plants**	Per Cent Plants Damaged by Wireworms**	Yield (Bus./A Shelled Corn)
Aldrin—Fall	299	.03	48.5
Aldrin—Spring	262	.08	49.3
Heptachlor)—F.	246	.12	47.6
Heptachlor—Sp.	283	.07	48.9
Untreated Check	214	7.00	34.3

\*Applied at seven to ten days before planting.

\*\*In 50 five-foot samples of row.

TABLE II—Summary of Cutworm Control in Soil "Broadcast" Treatment Tests, Carteret County, North Carolina—1955.

Treatment (2 lbs./A. of Active Ingredient)	Per Cent Plants Damaged
Aldrin	39
Chlordane	93
Dieldrin	53
Heptachlor	32
Untreated check	88

TABLE III—Summary of Billbug Control in Soil "Broadcast" Treatment Tests, Craven County, North Carolina—1955.\*

Per cent of Plants Damaged by Billbugs			
Aldrin	Dieldrin	Heptachlor	Check
—	13	—	60
1	—	1	8
—	3	—	70
—	3	—	50

\*Applied at seven to ten days before planting.

TABLE IV—Summary of Plant Counts in 1956 Corn in Plots Treated with Dieldrin in March 1955, Craven County, North Carolina, for Billbug Control.

	May 25th		May 31st	
	Total Plants	Per Cent Plants Injured by Billbugs	Total Plants	Per Cent Plants Injured by Billbugs
Field No. 1	66	13.6	54	11.1
Check	59	27.1	48	31.3
Field No. 2	88	37.5	87	29.9
Check	87	66.7	72	59.7

Corn is in a serious competitive situation in North Carolina as regards its agronomic economy. In the tobacco growing regions it does not command the care in planting and cultivation that tobacco does. Many times, corn is planted on the poorer soils, the better soils being reserved for tobacco. On some farms, there is a recognizable soil pest problem in certain areas, such as poorly drained fields, near ditch banks, or in low spots of the field. The practice of growing corn after corn increases the pest problem and is especially ill-advised where billbugs are present. Under such conditions, a preventive program of soil broadcast treatment is highly recommended. We have used various materials in the broadcast method, including aldrin, chlordane, dieldrin, DDT, endrin, heptachlor, Systox, and others. Under our conditions of heavy wireworm infestations, we have found chlordane and DDT to be too slow to protect the corn if applied just before planting. The preferred materials for control of wireworms and billbugs are aldrin, dieldrin, and heptachlor, applied at the rate of two pounds of active ingredient per acre either as a dust or as a granular formulation. Under field conditions, we expect two to three years protection against wireworms.

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# Considérations sur l'Emploi de l'Heptachlore Utilisé en Traitement du Sol

Par J. LHOSTE, J. D'AGUILAR, et J. L. GÉRARD  
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## ABSTRACT<sup>1</sup>

*Experiments in France using technical heptachlor for control of wireworms of the genus Agriotes gave the following results:*

1. SOIL TREATMENT. Heptachlor at 4 kg. per hectare gave better control of wireworms on potatoes than did aldrin at the same rate.
2. SEED DRESSING. Better results were obtained with aldrin at 17 g. than with heptachlor at 25 g. per 100 kg. wheat. But both products are good seed protectants against wireworms.
3. PERSISTENCE OF THE INSECTICIDES IN THE SOIL. Up to the sixth month after treatment the original amounts of heptachlor, dieldrin, and aldrin, were found in the soil. A year after treatment, only one-tenth of each insecticide was left in the upper 6 cm. of soil.
4. INSECTICIDE RESIDUES. The residues in vegetables grown in treated soil were determined by biological methods. Less than 0.1 ppm of heptachlor, aldrin and dieldrin, was found in radishes and carrots; no residue was found in potatoes.

<sup>1</sup> This paper was published in C.-R. Acad. Agric. de France, Séance du 28 novembre, 1958.





# Tests of Fertilizer-Heptachlor Mixtures in Italy

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The studies on the preparation of insecticide-fertilizer mixtures in Italy were begun in 1953 and the large field tests, in early 1954.

The insecticide compounds tested in combination with fertilizers were BHC, chlordane, aldrin, and heptachlor, especially aldrin and heptachlor.

Heptachlor has quickly become very popular in our country for its persistence and for its stability in the lightly alkaline soils. Heptachlor, moreover, neither imparts any objectionable flavour and odour, nor causes any morphological or physiological change in tobacco and other crops that are susceptible to BHC, lindane, and chlordane.

This paper reports results obtained in Italy in tests conducted on various crops with granulated superphosphate (18–20% of phosphoric anhydride) mixed with 0.5% heptachlor.

## POTATOES

The tests were conducted in many middle and northern districts of Italy, i.e.: Parma, Sondrio, Modena, Trento, Novara, Bologna, Viterbo, L'Aquila, etc.

Each test was composed of three main treatments, as follows: (a) replicated plots were treated with superphosphate mixed with heptachlor at the rate of 4 qls.; (b) replicated plots were treated with standard superphosphate at the rate of 4 qls. per hectare, and after six days were treated again with 6% heptachlor dust at a rate equivalent to 2 Kg. of technical heptachlor per hectare; and (c) replicated check plots were treated with standard superphosphate at the rate of 4 qls. per hectare.

All the tests were successful: in the (a) and (b) plots complete control was obtained of wireworms, mole crickets, and adults of the Colorado potato beetle, whereas all the untreated check plots were severely infested, showing 25–60% injury. The insecticidal action of the mixture persisted more than 60 days after treatment. The difference between the (a) and (b) treatments was not significant.

## SUGAR BEETS

In Italy the fertilizer-insecticide mixtures have been largely used in the sugar beet area; in this country the sugar beet crop is extremely important and is severely damaged by the soil pests *Agriotes*, *Cleonus*, *Lixus*, etc. The tests were therefore conducted in every Italian sugar beet growing district. The Experimental Station of Rovigo completed a large-scale field test in the Veneto sugar beet area, employing super-phosphate mixed with heptachlor.

Each test was composed of three main treatments similar to those employed for potatoes, as follows: (a) replicated plots were treated with superphosphate mixed with heptachlor; (b) replicated plots were treated with standard superphosphate, and after six days were treated once more with the heptachlor dust; and (c) replicated check plots were treated with standard superphosphate.

The tests showed that complete control was obtained of all the various pests in the (a) and (b) plots, and the Experimental Station of Sugar Beet Culture of Rovigo stated that from the results obtained in the different trials it is evident that the superphosphate-heptachlor mixture has attained its purpose and has completely controlled the soil infestations; also, that this treatment is cheaper than when the insecticide is applied after the fertilizer.

From the trials carried out by the Experimental Station of Rovigo, and the other trials, it was evident that the insecticidal action of the mixture persisted for almost 80 days after the treatment.

## TOBACCO

The tests were conducted in 1955 in some districts of Toscana and Umbria.

In these trials, conducted with the purpose already mentioned, complete control of "*Agriotes lineatus*" was obtained in the (a) and (b) plots, the differences between the two

not being significant. The test also made evident the saving of time and money resulting from broadcasting the fertilizer and insecticide in one operation; it has, moreover, confirmed the effectiveness of heptachlor against tobacco pests and its property of not imparting any off-flavour to this crop, nor altering in any way its morphological or physical features.

### TOMATOES

This crop is very important because it supplies the canning industries, so widespread in many districts of northern, middle, and southern Italy. We, therefore, did our best to test heptachlor activated with superphosphate on the tomato crop.

Trials were carried out in each representative district: Parma, Piacenza, Perugia, Napoli, Salerno, etc. The tests confirmed all previous findings and "*Agriotes lineatus*" was completely controlled in the (a) and (b) plots. It was interesting to note that in many tests heptachlor mixed with superphosphate as in the (a) treatment showed a slightly stronger and more persistent action than in the (b) treatment: the insectidal action persisted in the first case for 60–80 days, whereas, in the second, it did not exceed 40–60 days.

### CONCLUSIONS

From the results obtained in the numerous tests of superphosphate mixed with heptachlor, summarized in this paper, it is evident that applying the right insecticides and proper fertilizers to the soil in one operation solves an important economic problem and results in a considerable saving. The saving is equal to one-half the cost of making the two tillage operations separately.

Tests showed that the suitable rates of granulated superphosphate (18–20% of  $P_2O_5$ ) mixed with 0.5% heptachlor, are 4 qls. per hectare, equivalent to a rate of 2 Kgs. of technical heptachlor per hectare.

# Control of Carrot Fly (*Psila rosae* F.) with Chlordane in Holland

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## ABSTRACT

The damage caused by the carrot fly, *Psila rosae* F., in several parts of the Netherlands has been so severe that control has become absolutely necessary. An effective method has been developed which is now generally applied by the growers of carrots.

Of several insecticides tested, chlordane has been the most satisfactory.

The carrot fly has been a rather serious pest in various carrot growing centres of Holland. The damage was so severe locally that it was no longer remunerative to grow carrots.

Two categories of carrots are known: (a) the short-growing, slender carrots (type Amsterdamsche Bak or Nantes), which are considered as rather delicate vegetables; and (b) the long-growing, coarser winter carrots (type Flakkeese or Berlikummer), which are used in Holland for consumption on a moderate scale.

The short-growing carrots have a growth period of some three months; the long-growing ones generally develop in about six months. The latter type suffers most from carrot fly damage because of its longer growth period, and a good method for controlling the insect is essential.

The adult flies are present in the carrot fields for extended periods and because the toxicities of the insecticides used to control the insect were short-lived, several applications had to be made every season. This method of control was not economical and attempts have been made to find insecticides which would be toxic for a longer time.

Chlordane was found to give improved control of the carrot fly in Holland, and a larger output of sound carrots was obtained. When the insecticide gave only moderate protection, the reason, in most cases, could be attributed to an insufficiently fine distribution of the actual ingredient into the soil (see also Table II). Chlordane is still being used in Holland in 1956, although cheaper products are in competition with it.

In Switzerland, Giinthart (1950) obtained good results in 1947 with an emulsifiable oil of chlordane against the carrot fly and other soil insects. In 1949, Maag (1949) published a pamphlet in Switzerland with directions for using chlordane emulsifiable oil against the carrot fly. He recommended using 300–400 ccs. of the material per 300–400 litres of water per acre, (0.5–0.7 pints per 66–83 Imperial gallons of water per 1/40 acre).

In Holland, we have confirmed these results with chlordane. We use the insecticide as a dust and the results obtained are as good as those obtained with the emulsifiable oil. In our experiments the insecticide was applied by broadcasting the dust over the soil surface. For an even distribution, the dust was first mixed with some moist sand, broadcast, and then worked into the soil with a fork or rake to a depth of not more than 5 cm. (2 inches).

Several experiments were conducted on short-growing carrots in 1951, 1952, and 1953, using this application method. The results obtained are given in Table I.

In the 1951 test, a dosage of 3 grams actual ingredient per m<sup>2</sup> (0.1–0.15 oz. per square yard) was used. Although the dosages were reduced to 1.0–0.75 grams (0.03–0.025 oz. per square yard) in the 1952 and 1953 tests, satisfactory control of the carrot fly was still obtained. Consequently, the recommended dosage for short-growing carrots is now 1 gram actual ingredient per m<sup>2</sup>.

In 1952, an experiment using chlordane dust worked into the soil before planting was conducted on short-growing carrots at Sloten and Den Dolder. These carrots were harvested and inspected for carrot fly damage in the early summer; the results obtained are given in Table II. The land at Sloten was fraised (with a rotary hoe) and was spaded at Den Dolder. More carrots were sown on this land immediately without any additional insecticide being applied. These carrots were harvested in late summer and the information obtained on carrot fly injury is given in Table III.

TABLE I—Control of the Carrot Fly on Short-growing Carrots with Chlordane Dust Applied to the Soil Before Planting, 1951–1953.

1951			1952			1953		
Object	Quantity actual ingredient	Percent-age of infest-ation	Object	Quantity actual ingredient	Percent-age of infest-ation	Object	Quantity actual ingredient	Percent-age of infest-ation
Untreated Chlordane	Arnhem 3 g/m <sup>2</sup>	70 9	Untreated Chlordane	Arnhem I	57,2	Untreated Chlordane	Arnhem I	46,8
				3 g/m <sup>2</sup>	0,5		1,5 g/m <sup>2</sup>	2,5
				1.5 g/m <sup>2</sup>	2,1		0,75 g/m <sup>2</sup>	3,8
			Untreated Chlordane	Arnhem II	35	Untreated Chlordane	Arnhem II	27,5
				1 g/m <sup>2</sup>	4,5		0,75 g/m <sup>2</sup>	3,75
			Untreated Chlordane	Eindhoven	16,9			
				1 g/m <sup>2</sup>	0,25			

TABLE II—Control of the Carrot Fly on Short-growing Carrots in Early Summer, 1952.

Object	Quantity actual ingredient	Percentage of infestation	Object	Quantity actual ingredient	Percentage of infestation
Sloten I (spring-summer)			Den Dolder I (spring-summer)		
Untreated Chlordane	4 g/m <sup>2</sup>	12 3,6	Untreated Chlordane	1,5 g/m <sup>2</sup>	86 10

TABLE III—Control of the Carrot Fly on Short-growing Carrots Planted on Land that Had Previously been Planted to Carrots (Table IV) and Without Further Insecticidal Treatment, 1952.

Object	Quantity actual ingredient	Percentage of infestation	Object	Quantity actual ingredient	Percentage of infestation
Sloten II (summer-autumn)			Den Dolder II (summer-autumn)		
Untreated Chlordane	4 g/m <sup>2</sup>	11,7 0,3	Untreated Chlordane	1,5 g/m <sup>2</sup>	37,8 1,4
(only treated for test I in spring)			(only treated for test I in spring)		

Reasonably good protection was obtained on the two successive carrot crops with only one soil application of chlordane. The toxicity of this material to the carrot fly lasted at least 6–7 months. The control obtained in the second test at Sloten and Den Dolder was better than that obtained in the first test although the insecticide had been in the soil for at least three months. These excellent results are due to the tillage (fraising and spading) which gave a better distribution of the insecticide into the soil. On the basis of these results fraising is now recommended.

In 1952, experiments using different application rates of chlordane were conducted on long-growing carrots at Beetgum and Berkhout. An additional test was conducted at Beetgum in 1953 when only one application rate was used. The results obtained from these experiments are given in Table IV.



TABLE IV—Control of the Carrot Fly on Long-growing Carrots with Chlordane, 1952–1953.

Beetgum 1952.			Berkhout 1952.				
Object	Quantity actual ingredient	Percentage of infestation	Object	Quantity actual ingredient	Percentage slight infestation	Percentage heavy infestation	Percentage total infestation
Untreated		44,75	Untreated		4,7	94,5	99,2
Chlordane	3 g/m <sup>2</sup>	1,5	Chlordane	1 g/m <sup>2</sup>	30,2	20,2	50,4
Chlordane	1 g/m <sup>2</sup>	9,5	Chlordane	2 g/m <sup>2</sup>	25,7	10	35,7
			Chlordane	4 g/m <sup>2</sup>	22,0	5	27

Beetgum 1953.				
Object	Quantity actual ingredient	Percentage slight infestation	Percentage heavy infestation	Percentage total infestation
Untreated		18	43	61
Chlordane	2 g/m <sup>2</sup>	6		6

It appears from the results that when infestations of the carrot fly are moderately heavy, 45%–61%, good control can be obtained with a soil treatment of 2 grams actual chlordane per m<sup>2</sup> (0.06 oz. per square yard). When the infestation is heavy, 90%–100%, as was present at Berkhout, a dosage of 4 grams actual chlordane per m<sup>2</sup> (0.13 oz. per square yard) is necessary. With this dosage, only 5% of the carrots were heavily infested.

The recommendations for the control of the carrot fly on long-growing carrots based on these and other results reads as follows: In as far as the expected infestations will locally be moderately heavy, 2 grams actual ingredient per m<sup>2</sup> (0.06 oz. per square yard) should be applied; if a more serious infestation is expected, it is desirable to increase the dosage to 4 grams actual ingredient per m<sup>2</sup> (0.13 oz. per square yard). The application should be hygienically justified, not only for the grower, but also for the consumer.

In Holland, it is obligatory to print directions for the use of any insecticide on the package containing the material. Information is also given to the grower by Government Services, such as Horticultural and Agricultural Information Services, and the Plant Protection Service.

The hygienical measures necessary to protect the consumer are directed by the Government Institute for Public Health. This institute has determined that carrots treated with chlordane in dosages given in the above recommendations, are not harmful to the consumer.

A number of taste tests on carrots grown on chlordane-treated soil have been conducted in cooperation with the Institute for the Conservation and Preservation of Horticultural Products (Instituut voor Bewaring en Verwerking van Tuinbouwproducten) at Wageningen. The tests were conducted on carrots treated with such dosages of chlordane as are usually applied in practice, as well as with larger and smaller dosages. No positive influence on taste could be determined. There is, therefore, no objection to the use of chlordane on carrots if it is applied at the recommended rates.

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# Estimation of Rice Losses Caused by the Rice Stem Borer

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## ABSTRACT

The rice stem borer, *Chilo suppressalis* Walker, is one of the important pests of the rice plant in Japan, and thus an adequate method of estimating the loss of rice caused by this insect is urgently needed.

Difficulties are encountered in sampling methods due to the variable nature of an infestation within a field, between fields, and between sections in a given area. This unequal distribution is due partly to the behaviour of the stem borer and partly to the heterogeneous nature of the paddy fields in Japan, arising from the use of intensive cultural methods.

A comparison of the grain yields from infested or healthy stems or hills of rice in a crop occasionally fails to give a satisfactory loss estimate when the infestation is slight. In such an infestation, the stem borer usually attacks the stems or hills showing the greatest vegetative activity; thus, the estimate must be calculated from the figures referring to both the degree of infestation and the yield of rice determined for each of the selected groups of hills.

Rice is the most important crop in the agriculture of Japan, and the rice stem borer, *Chilo suppressalis* Walker (= *C. simplex* Butler), is the most destructive insect attacking grain in the country. The annual loss attributable to the attack of the second brood of this stem borer was once roughly estimated to be about five per cent of the total rice production of the country, but no really satisfactory method of estimating the loss has yet been devised.

The stem borer has two broods a year throughout most of its distribution area, the first-brood larvae attacking the rice seedling while it is in the nursery bed and for some time after transplanting into the paddy field. The rice plant normally produces an excess of tillers, the later appearing ones never being productive; the productive tillers of an infested plant have a lower yield, although the actual non-infested tillers increase the grain productivity per head. Additionally, to compensate for losses the normal late appearing non-productive tillers may survive and head. The second-brood larvae attack the plant in the period between heading and harvesting, causing the appearance of "whiteheads" (white unfilled grains) and poor and uneven maturing of grains.

Developing a practical method of estimating yield losses by these attacks is beset by many difficulties due to the habit of the stem borer and the peculiarities of farming conditions in Japan. The stem borer moths prefer to oviposit on the host plant and moths of the first brood choose rice that was transplanted early into the paddy field. With a lower rate of seeding in the nursery beds, ample quantities of fertilisers and restricted watering, the seedlings, after transplanting into the paddy field, develop their root structures very quickly; generally speaking, transplanting is conducted around the date of peak appearance of first-brood moths and thus, these well established plants are liable to receive more egg masses.

The high proportion of first-brood moths appearing before transplanting, do not oviposit satisfactorily in the nursery bed and the hatching larvae easily succumb, giving high mortality counts. Later moths appearing after transplanting, oviposit on the seedling transplants more easily and the ensuing larvae, hatched in the paddy field, survive more easily under the favourable host plant growth conditions. Therefore, the infestation of the first-brood larvae in the paddy field can be attributed mainly to oviposition occurring after transplanting, the intensity of infestation generally being highly dependent on the date of transplanting.

Ovipositing moths of the second brood also prefer rice plants exhibiting vigorous growth and choose plants that are strongly coloured as a result of adequate amounts of fertiliser having been used in the formative period of the head; they also choose later varieties rather than earlier ones. Mortality figures for larvae that have bored into the stem show remarkable variations for different rice varieties, this affecting the intensity of infestation.

Farming in Japan is very intensive and about two-thirds of the farmers cultivate less than 1 Cho (approximately equal to 1 hectare or 2.47 acres). A paddy field is usually as small as 0.1 Cho or less, and many varieties are grown even within a narrow boundary to lessen the risk of climatic and other environmental abnormalities. The treatment of the fields and the rice plant cultural practices differ between farmers; these differences intensify the mosaic features of the fields and the distribution of stem borer infestations.

For example, a survey made over 77.6 Cho of rice fields in Tateyama City, Chiba Prefecture, revealed that there were 17 varieties growing in 206 rice fields in which transplanting began May 10 and continued until June 20. The number of affected stems per hill at the end of the first-brood infestation ranged from 0.05 to 2.13; this may be rather an exaggerated case, but the general pattern of the distribution of an infestation over a narrow area is well shown. It follows from this that in a specified area, sampling rice fields to estimate crop losses is very difficult; but classifying the fields into categories correlating the occurrence and intensity of the infestation helps to give a more precise estimation.

However, the distribution of the infestation within a rice field is not uniform; stem borer moths deposit eggs in definite masses, preferring not only strongly growing fields as a whole but individual vigorous plants within the field. The newly-hatched larvae enter the rice stems gregariously in the initial stage but later disperse. Hence, the infestation generally occurs in patches in a field, centering on hills on which the egg masses were deposited and dispersing radially. The larvae of the first brood disperse more actively than those of the second brood, and the distribution of the infestation is more uniform in the first brood than in the second brood. Generally, the infestation is less at the perimeter than at the centre of a field.

In most fields, rice plants are transplanted as hills at almost equidistant spacing and thus, by systematically sampling rice hills at constant intervals from several hills chosen at random over a field, the least biased estimate of the infestation is given. Sampling hills successively from several hills chosen at random over a field can also give a fairly good estimate, especially when the hills are sampled in a direction diagonally opposite to the planted rows, but this method is rather more laborious than the former. Sampling the given number of hills collectively in a quadrature or on a line, frequently used in estimating rice yields, entails less labour than the two methods mentioned above, but is prone to give a higher estimate as far as infestations of the rice stem borer are concerned.

Thus, an infestation spreads contagiously and the variance of the estimate is exceedingly large, even if the hills are sampled systematically; if the infestation is slight, it is necessary to sample several hundred hills in a field to obtain a reasonably accurate estimate.

Formerly, the losses of rice due to this stem borer had been evaluated from the yields of infested and uninfested stems; for example, the Ministry of Agriculture and Forestry once used, though tentatively, a method of estimating losses attributable to the second brood by identifying the infested and uninfested stems and then calculating the rate of loss in the following way:

Total yield of rice from all uninfested stems divided by the number of uninfested stems = Average yield of uninfested stems. Average yield of uninfested stem multiplied by the number of infested stems = Potential yield of infested stems if they were free from infestation. Yield of all uninfested stems plus potential yield of infested stems if they were free from infestation = Standard yield when all stems are free from infestation.

Difference of standard yield when all stems are free from infestation and the total yield of all infested and uninfested stems divided by standard yield = Rate of loss.

This method can only be expected to give a satisfactory estimate when there is no compensation between the infested and uninfested stems of a hill and the infestation occurs at random concerning the hills and stems. However, with both first and second broods, hills showing a high production of stems are attacked more frequently.

Stems bearing larger heads are more likely to be attacked. For example, in Okayama Prefecture, measurements of the length of head, variety Toyoho, showed that the average length of the heads of infested stems was 20.1 cm., while that of the uninfested stems was 19.1 cm., a difference of 1.0 cm. Therefore, if the above-mentioned method is adopted, the potential yield of infested stems is underestimated and hence, the rate of loss is also under-



estimated. A survey by this method on the losses of rice caused by the stem borer occasionally gives a negative value for the rate of loss when the infestation of the second brood is slight.

Thus, it is necessary to estimate the rate of loss from groups of hills by the relation between the intensity of the infestation and the yield. Generally, a close negative correlation can be observed between the intensity of the infestation, say the percentage of infested stems, and the yield of groups of hills reaped from the same field. If such a correlation exists in most cases, the potential, or standard yield will be obtained by making use of the empirical formula between the intensity of infestation and the yield, indicating the infestation as nil. Whether the relation is rectilinear or curved is not fully clarified, but practically it can be considered rectilinear. The rate of loss is calculated by the ratio of the difference of actual and standard yields of the field to the standard yield obtained in this way. It can be more directly calculated by multiplying the percentage of infested stems by a fixed constant.

This method of estimation has some restriction in general use, since the relation between intensity of infestation and yield differs to some degree between fields for many reasons. In the first-brood attack, the secondary or compensatory reaction of the rice plant affects the actual reduction of rice yield; and this is much influenced by the variety, care of the crop after the infestation occurs, soil fertility, and climatic conditions during the year. In the second brood the period of attack in relation to maturity of the grain has much influence on the ripening of the grain; the same intensity of infestation expressed as percentage of infested stems shows lower losses in early varieties than in late varieties. But this method is convenient because the loss can be roughly estimated quickly by multiplying a constant by the intensity of infestation, say the percentage of infested stems, which can be determined with far less labour than the yield of infested and uninfested plants in the field. With late varieties, which are most important in loss estimation because the infestation is severe, losses due to the first brood can be roughly estimated by multiplying 0.5 by the percentage of infested stems at the end of the first-brood attack. The losses attributable to the second brood can be quickly estimated by multiplying 0.4 by the percentage of infested stems at harvest.

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# Control of *Pomacea (Ampullaria) lineata* Snails in Rice Fields

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## ABSTRACT<sup>1</sup>

In Surinam, the snail, *Pomacea (Ampullaria) lineata* Spix, is regarded as the most serious menace to the rice crop. *P. glauca* L., living among *P. lineata*, constitutes only a low percentage of the total snail population. This situation has, in part, been brought about by the recent extension of the practice of direct sowing in the field instead of transplanting from nursery-beds.

The author gives morphological recognition characters for both molluscs, and deals with their life histories.

Results of laboratory experiments with four chemicals, viz. BHC 5% dust, BHC 25% w.p., copper sulphate, and colloidal copper paste 20%, against *P. lineata* in glass cylinders are discussed. Medium sized and full-grown molluscs showed far greater susceptibility to BHC than the newly-hatched molluscs; the difference in susceptibility to the coppers was very slight. The relation between LD 90 in kg/ha and water depth in cm for BHC and copper sulphate against newly-hatched molluscs was nearly linear, and resulted in the following control rates: 1 kg BHC/ha/cm water height; 100 gram copper sulphate/ha/cm water height.

Tentative control recommendations are given for each of the three rice cultivation practices now in use in Surinam, viz. 1, direct, sowing in water, 2, direct sowing on muddy fields, and 3, transplanting from nursery-beds to the fields.

<sup>1</sup> Full article published in Agr. Expt. Sta. Surinam Bull. 68, 1956.





# Animals Affecting Cultivated Mushrooms<sup>1</sup>

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## ABSTRACT

*This paper gives short accounts of the biology and mode of action of the important animal pests affecting cultivated mushrooms, and the present status of their control.*

*Sixty per cent of the mushroom industry in the United States is centred in Chester, Berks, and Delaware counties in Pennsylvania, with lesser areas in Butler and Union counties; in the Hudson River Valley in New York State; in Ohio, Michigan, Illinois, Missouri, California, and Washington.*

*Cultivated mushrooms in the United States are subject to injury by species of at least three families of Diptera (Mycetophilidae, Cecidomyiidae, and Phoridae); by several species of Collembola or springtails; by mites of the genera Tyroglyphus, Rhizoglyphus, and Pigmaeophorus; and by several kinds of nematodes (Rhabditis, Ditylenchus, etc.). Preventive and control measures now in use include composting; steam; soil fumigation; pyrethrum dusts, sprays and aerosols; and DDT, lindane, malathion, etc. as sprays and dusts.*

The mushroom industry in Pennsylvania represents about 60 per cent of the entire production of cultivated mushrooms in the United States. The annual production in Pennsylvania is valued at more than 15 million dollars, more than that of several other major crops.

During a thirty-year study of the animal pests of this crop, a considerable number of animals of various kinds have been found in mushroom houses and in mushroom compost outdoors. Fortunately, only a small proportion of these species are injurious to cultivated mushrooms and mycelium. These are briefly discussed in this paper.

Nematodes, mites, springtails and flies may enter mushroom growing houses in a number of ways: by crawling into the compost pile from the soil beneath it and then being carried into the house in the compost when the beds are filled; by being carried into the beds in the casing soil; and by flying or crawling through the ventilators and doors. Prevention and sanitation, as described later, are therefore, of importance in keeping these pests from entering the house.

Once within the house beds, each kind of pest causes its own type of injury and must be handled in a specific manner.

## NEMATODES

Nematodes or "eelworms", while not always the most important mushroom pests, have in recent years caused considerable injury to the mushroom industry. Several species occur in mushroom beds, one group, *Ditylenchus* species, with a tiny stylet-like mouthpart pierces the mushroom tissues and mycelium, thus causing direct injury. The other group, *Rhabditis* species, does not have piercing mouthparts, hence cannot puncture the mycelial cells. Both kinds, however, carry myriads of bacteria and mold spores as they crawl through compost and over the mushroom surfaces. They can thus spread several mushroom diseases. In the places in the compost where the nematodes are abundant, bacteria are usually also abundant, the compost is moist and has a distinct odor. Here the mycelium has disappeared and mushrooms no longer appear on the soil above these spots.

These nematodes enter the mushroom house either in the casing soil or in the compost. Once in the beds, they may escape the pasteurizing heat by being inside cooler wet lumps, large corn cob pieces, or in the surface of the bed boards. Between crops they can survive drying out in the board surfaces by "anabiosis".

The controls, therefore, are to thoroughly compost on a concrete floor to prevent the nematodes from entering the compost; breaking up all lumps when filling the beds, and thorough and uniform pasteurization of the beds to 140° F. (although these nematodes die at less than 130° F. for one-half hour); steaming or gassing the casing soil with chloropicrin; and steaming the empty house between crops to kill the nematodes in the board surfaces which should be previously dampened to activate them.

<sup>1</sup> Paper No. 2084 in the journal series of the Pennsylvania Agricultural Experiment Station.

## MITES

Mites of several species are found in mushroom beds. *Tyroglyphus*, *Rhizoglyphus*, and *Histiostoma* mites are small, glistening white, oval and slow crawling. They feed on molds, decaying fungi and other organic materials outdoors, and enter the houses in the compost. In the beds they feed to some extent on the mushroom mycelium and occasionally eat into the grains of grain spawn, but prefer certain molds. On the beds they feed on older or diseased mushrooms and the Tyroglyphid and Rhizoglyphid mites may chew tiny slimy holes into the mushrooms. If they eat the spawn pieces, the grower may drench with nicotine solution, but it is sometimes necessary to respawn midway between the infested spawn pieces, leaving the latter in place as food for the mites. These mites have a "hypopus" stage which can be spread by attaching themselves to flies, baskets and pickers.

Two mites which have been seen causing injury only rarely in Pennsylvania are *Linopodes antennaeipes* and a *Tarsonemus* species. These feed on the base of the mushroom, turning it red, and on the small buttons. *Linopodes* is susceptible to nicotine dusts.

*Pigmaeophorus americanus*, the so-called pigmy mite, is reddish with a whitish stripe down the back, and is very tiny and active. These mites frequently pile up on the casing soil and on the mushrooms in enormous numbers. Most of the life-cycle is spent down in the compost where they feed mostly on *Chaetomium*, *Penicillium* and other molds; very little feeding is done on mushroom mycelium. The greatly swollen female mite may lay 50 or more tiny spherical eggs, which hatch in about 24 hours into minute white 6-legged larvae. These feed on the molds and develop through two 8-legged nymphal stages to the adult. The entire cycle may be completed in seven days. It is clear, therefore, that even a few of these mites escaping the pasteurizing heat can increase to enormous numbers by the time the first mushrooms appear. They are then so overcrowded in the compost mold that they migrate to the surface to find new food supplies. They can crawl considerable distances to find new mold. They have been more abundant in synthetic composts than in manure composts because of molds.

These mites carry bacteria and spores of mushroom mold diseases. They also detract from the appearance of fresh mushrooms in the basket. Cannermen of mushrooms can wash them off before they get into the cans, but soup processors have considerable difficulty eliminating them. Also, some pickers are definitely allergic to these mites.

Every attempt should be made to prevent these mites from getting started in the beds. This means prevention by composting on clean concrete if possible; scraping and cleaning up soil and old mushrooms close to the composting grounds; short but complete composting to break up lumps and to get uniform heat throughout the pile. In the compost in the beds there should be no wet lumps or large cobs within which mites may escape the heat. A system of pasteurization should be used (including steam if necessary) to prevent growth of *Chaetomium* and other molds in the compost; ventilation at intervals during the pasteurization may aid in this. Sterilization of the casing soil is also important.

Several of the miticides used against red spider mites in greenhouses are also quite effective against the *Pigmaeophorus* mites, but since these are poisonous, and as we do not yet have a tolerance set on them for use on cultivated mushrooms, we cannot recommend them in Pennsylvania.

Frequently numbers of active gamasid mites (*Parasitidae*), or "red spiders", are seen running rapidly over the beds. These are much larger than the *Pigameophorus* mites and may annoy the mushroom pickers by crawling over exposed skin surfaces, but they do not seem to cause noticeable damage to mushrooms or mycelium. Instead, they feed on smaller mites, springtails, fly larvae and nematodes. If too annoying, nicotine dusts and sprays will kill them.

## SPRINGTAILS

Springtails (*Collembola*) are tiny active gray insects which formerly chewed the newly planted spawn pieces and ate holes in the mushrooms. They are now scarce, due chiefly to raising the bottom beds to obtain a good heat in the compost: also to the use of nicotine dusts and sprays.

## FLIES (DIPTERA)

Up to the end of World War II, sciarid fly or mushroom fly was considered to be one of our most important mushroom pests, as the black-headed larvae caused much injury

by destroying the mycelium in the compost, and by burrowing up the mushroom stem into the cap, making these mushrooms unfit for canning or for the fresh market. Since the advent of DDT sprayed on the mushroom house walls, however, they have become a minor pest in Pennsylvania, and are no longer of importance to our canners. Pyrethrum compounds also are of value against this fly.

The small active black phorid fly or manure fly often become abundant in mushroom houses especially at the end of the spring crop in May and June. Their larvae, white but without the black head capsule as in the *Sciara* larva, feed on the mycelium in and near the spawn pieces. In Pennsylvania, they usually feed on the mushrooms only in the warm weather of late spring; then the eggs may be laid among the gills of open mushrooms and the flies may appear in enormous numbers, leaving the mushroom houses and invading nearby residences, much to the annoyance of the inhabitants. Some persons are actually allergic to these flies.

Pyrethrum dusts do not kill these flies as easily as they do the *Sciarid* flies. Lindane compounds, Methoxychlor and DDT are still effective against Phorid flies in some houses; in others there is apparent resistance and poor control by these chemicals. A 4 per cent Malathion dust has proved to be very effective against these flies, but has only a short residual life of two or three days at most in the mushroom house. Diazinon is also very toxic to them, and as a treatment for walls, doors, ventilators, attics and other interior surfaces of the houses, has a much longer residual life than Malathion. However, neither Malathion nor Diazinon have yet been given a tolerance for mushrooms by the United States Food and Drug Administration. Therefore, they cannot be recommended for dusting on the beds until those tolerances are announced.

The very small orange and black cecid flies or midges are only occasionally seen in mushroom houses, although the tiny orange to whitish larvae are sometimes very abundant, feeding on the mycelium, burrowing just under the skin of the mushroom stem, and especially in among the gills, where it is practically impossible to eliminate them during the canning process. Hence, such mushrooms must be discarded and destroyed, which is a considerable loss in the event of a heavy infestation of these larvae.

Fortunately these insects have thus far appeared in only an occasional Pennsylvania mushroom house. When they occur, they increase rapidly, for the larvae are paedogenetic, i.e., the larvae can produce new larvae without going on to the pupa and adult stages. The flies, if present, are easily killed by pyrethrum dusts or sprays or aerosols, but the larvae are less affected by these insecticides. Hydrated lime, ground limestone and nicotine dusts help to dry up the larvae if the soil is not wet, but new larvae are constantly appearing from down in the beds. Close cleaning of the beds and destruction of infested mushrooms also helps to reduce their numbers, as they move over the soil from mushroom to mushroom. Diazinon dusts and sprays also kill them, but as noted under the Phorid fly discussion above, this material does not yet have a tolerance assigned to it.

### CONCLUSION

In brief, then, the following are general rules to follow in handling the mushroom animal pests:

1. Sanitation. Clean up thoroughly around the mushroom houses; don't allow old compost to lie around to breed flies and other pests and diseases. Remove it far from the premises. Destroy all butts, stems, and so forth, as soon as removed from the houses.

2. Composting. Compost on a concrete floor which is tight and well drained. Don't allow any drainage onto this floor. Get complete but rapid composting and break up all lumps and cobs; get the pile completely heated throughout. Do not allow time for a build-up of pests.

3. Pasteurization. In the beds, get uniform heating throughout the compost, up to 140° F. if possible in all beds, using steam if necessary. Ventilate at intervals during pasteurization to reduce build-up of molds and therefore of mites, and so forth.

4. Insecticides. Treat interior surfaces, walls, ceiling, ventilators, doors, attic, posts and outside of bed boards with DDT, lindane, methoxychlor or similar compounds as soon as the heat drops in the beds, before spawning if possible. Start dusting at once and do a thorough job at least once a week. However, do not allow heavy dosages of lindane to

settle on the producing beds, as the mushrooms may show a fairly high residue and a definite off-flavor when canned. The casing soil should be steamed or treated with chloropicrin or similar soil fumigant, being sure that no trace of these chemicals remains when the soil is placed on the beds.

5. At end of crop. Clean out the old compost thoroughly and remove it from the vicinity as soon as possible. Thorough steaming before the cleanout will destroy most of the fly stages, nematodes and mites. Diazinon, Malathion or other insecticides applied just before the cleanout will also keep down the fly population.

6. Between Crops. When the house is empty and the boards cleaned as thoroughly as possible, wet them on both sides if possible; this reactivates the nematodes which have become dormant in the board surfaces. After a day or two, give the house a good steaming to kill these nematodes. Formaldehyde used in the steam line at this time will also kill many mold spores present on the boards.



# Changes in Insect and Related Pest Fauna on Greenhouse Crops by Use of Organic Pesticides

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## ABSTRACT

By the application of modern organic pesticides, the species of insects, mites and related pests of ornamentals and vegetables in greenhouses of the Northeastern United States have been greatly reduced in number or severity of damage during the past 10 years. Many species of aphids, thrips, scales, roaches, weevils and other groups that normally live in the tropics and survive in the north only in greenhouses have been eliminated. Infestations of other pests such as leaf rollers, cutworms, green peach aphids, and onion thrips are just as completely destroyed by modern pesticides but they reinfest the greenhouse crops from out of doors and require periodic retreatment for continued control. Increase in flower production and quality has followed the elimination of pests.

Parathion is most widely used on greenhouse crops because it destroys more kinds of pests than any other chemical. Supplemental treatments include lindane for garden centipedes, metaldehyde for slugs and snails, sulfotepp or malathion for scales, DDT for cutworms and other pests, and Aramite, chlorobenzilate, demeton or schradan for resistant spider mites. The application of pesticides has been simplified by the use of aerosols containing parathion or other toxicant in methyl chloride, or of combustible powders as smokes.

Development of resistance among spider mites on roses and other crops to parathion and other acaricides is becoming more serious and widespread.

Pest control on vegetable and flower crops in greenhouses of the northern United States has become more efficient during the last ten years through the use of new pesticides and improved methods of application. Most pests that formerly seriously injured crops, as recorded by Gibson (1922), McDaniel (1931) and Weigel (1935), have either disappeared entirely in most greenhouses or have become extremely hard to find in an occasional greenhouse where control procedures have been lax. Pests still found in greenhouses fall into two groups. One group attack only plants native to the Tropics or the southern parts of this country and in the North survive only in greenhouses. Once they are eliminated from greenhouses the crops remain clean unless they are reintroduced on infested stock. The second group also infest outdoor crops or wild plants, and although the modern pesticides are just as effective against them reinfestations frequently occur which require seasonal treatments. Examples of the pests in each group are given below.

### GROUP 1

A carnation aphid (*Myzus polaris* Hille Ris Lambers)  
Crescent-marked lily aphid (*M. circumflexus* (Buck.) )  
Mexican mealybug (*Phenacoccus gossypii* T. & C.)  
Citrus mealybug (*Pseudococcus citri* (Risso) )  
An orchid thrips (*Taeniothrips xanthius* (Will.) )  
Greenhouse thrips (*Heliothrips haemorrhoidalis* (Bouché) )  
Fuller rose beetle (*Pantomorus godmani* (Crotch) )  
A black orchid weevil (*Diorymerellus laeviomargo* Champ.)  
Surinam roach (*Pycnoscelus surinamensis* (L.) )  
Tomato pinworm (*Keiferia lycopersicella* (Busck.) )  
Greenhouse leaf tier (*Udea rubigalis* (Guen.) )  
Chrysanthemum gall midge (*Diathromyia chrysanthemi* (Ahlb.) )  
Rose midge (*Dasyneura rhodophaga* (Coq.) )  
Soft scale (*Coccus hesperidum* L.)

### GROUP 2

Green peach aphid (*Myzus persicae* (Sulz.)  
Rose aphid (*Macrosiphum rosae* (L.) )  
Potato aphid (*M. solanifolii* (Ashm.) )

Melon aphid (*Aphis gossypii* (Glov.))  
 Onion thrips (*Thrips tabaci* (Lind.))  
 Flower thrips (*Frankliniella tritici* (Fitch))  
 The rose leaf rollers (*Platynota flavedana* (Clem.) and *P. stultana* Wlshm.)  
 Oblique-banded leaf roller (*Archips rosaceana* (Harr.))  
 Corn earworm (*Heliothis zea* (Boddie))  
 Cabbage looper (*Trichoplusia ni* (Hbn.))  
 Potato leafhopper (*Empoasca fabae* (Harr.))  
 Meadow spittlebug (*Philaenus leucophthalmus* (L.))  
 Tarnished plant bug (*Lygus lineolaris* (P. de B.))  
 Two-spotted spider mites (*Tetranychus telarius* (L.) and *T. cinnabarinus* (Boisd.))  
 Grape mealybug (*Pseudococcus maritimus* (Ehrh.))  
 Cutworms and leaf miners, several species.

### CONTROL ON FLOWER CROPS

The elimination of the many pests on greenhouse flower crops or their reduction to noninjurious numbers has come about through the use of new pesticides in the classes of chlorinated hydrocarbons or organic phosphorus compounds. These chemicals act both as immediate contact and as long residual poisons. The vapors of some compounds give an immediate kill, and in a greenhouse with closed ventilators vapors from residues also give a prolonged fumigating action. Some materials are systemic in action and may be applied to the soil, stems, or foliage.

In addition to dusts, wettable powders, and emulsion concentrates, most of these chemicals are formulated in aerosols and some in smokes or combustible powders and granules. Application in aerosols and smokes has increased their use in greenhouses because of the convenience and saving in labor.

Although a large number of these organic compounds are toxic to greenhouse pests, only certain ones have come into commercial usage on greenhouse crops, because of their low cost, ease in formulation, and safety to plants. Those most widely used will be discussed here. The dosages and methods of application are available from the manufacturer or in various publications (Smith *et al.* 1948, Gesell and Adams 1954, Kiplinger 1955, Porte and Smith 1955). It should be emphasized that every person who handles insecticides should follow the directions and heed all precautions on the container label.

Aramite is used for control of parathion-resistant spider mites. Chlorobenzilate, DMC, ovex, and Kelthane are used less extensively. Kelthane is toxic to mites showing resistance to other miticides, but resistance to Kelthane has been demonstrated. DMC and Kelthane are also toxic to cyclamen mites.

Chlordane is useful for the control of ants, crickets, grasshoppers, roaches, thrips, spittle bugs, white grubs, wireworms, and some species of slugs.

DDT controls thrips, cutworms, most leaf-eating beetles and caterpillars (except leaf rollers), sowbugs, centipedes, millipedes, chrysanthemum midges, rose midges, several leafhoppers, tarnished plant bugs, whiteflies, and most scales in the crawler stage.

Demeton and schradan, both systemic insecticides, are used in aerosols and sprays or in liquids for soil applications for the control of aphids and spider mites, including resistant strains. They are sometimes combined with Aramite or Chlorobenzilate to provide both systemic and contact actions against spider mites.

Dieldrin is toxic to thrips, grasshoppers, spittle bugs, white grubs, and wireworms.

Endrin in sprays is effective against the cyclamen mite and also against many other pests, but it will probably not be generally used in greenhouses because of its high toxicity to warm-blooded animals through skin contact. One should wear rubber gloves and tightly woven clothing while applying this material and while handling plants within 5 days after application.

Lindane is very effective against aphids, thrips, and spittle bugs on the foliage and against symphylids in the soil. It is combined with Aramite in aerosols for control of aphids as well as mites.

Malathion approaches parathion in its killing effect on various pests, but it is ineffective against leaf rollers on roses. It is also less effective against whiteflies and spider mites be-

cause of its shorter residual action. However, it is more toxic than parathion to aphids, flower thrips, mealybugs, and soft scales.

Metaldehyde in granulated or pelleted baits or in dusts, sometimes mixed with chlordane or calcium arsenate, is the most effective control for many species of slugs and snails.

Methoxychlor can be substituted for DDT against many pests, but its use in greenhouses is limited almost entirely to the control of two webbing spiders, *Erigone autumnalis* (E.M.) and *Eridantes eriginoides* (E.M.) on roses.

Parathion is toxic to a wider range of insects and related pests in the greenhouse than any other chemical tested (Smith, Lung and Fulton 1948) and is the most generally used insecticide on both ornamental and vegetable crops. It is toxic to certain snails, but is notably ineffective against slugs, soft scale, and false spider mites (*Brevipalpus inornatus* Banks and *B. australis* Tucker.).

Sulfotepp is used for the control of aphids, mealybugs, the soft scale, thrips and spider mites, including some resistant strains of two-spotted spider mites.

TEPP in aerosols is toxic to aphids, whiteflies, mealybugs, and nonresistant spider mites. Because its residual toxicity is short, it can be used on vegetable crops near harvest.

### PESTS REQUIRING SPECIAL TREATMENTS

Although most greenhouse pests are controlled by routine treatments with the various chemicals in sprays, dusts, or aerosols, a few species require special treatments.

Each spring during the 6-week migration period of the flower thrips great numbers of adults enter greenhouses through the ventilators and damage rose, carnation, and chrysanthemum flowers. Aerosol applications of malathion will destroy the thrips within the greenhouse. Coarse cloth screens treated with dieldrin or heptachlor and placed on the ventilators will prevent ingress of others (Naegle *et al.* 1955, Smith 1955).

Roses and some other crops have been severely damaged by the soft scale following the use of parathion aerosols, which eliminated parasites that had formerly kept the scale infestations in check. These infestations have been destroyed by aerosols containing malathion or sulfotepp.

Although cyclamen mites on snapdragons, chrysanthemums, African violets, and English ivy can be destroyed with parathion aerosols, some survive in badly distorted leaves and flowers of cyclamen, delphinium, and similar crops. Infestations on these crops can be eradicated by fumigation with methyl bromide (Smith and Latta 1939) or immersion in hot water (Smith 1933). Repeated sprays with emulsions of DMC or endrin have recently destroyed infestations on crops where neither parathion aerosols nor fumigation can be used (Morishita and Jefferson 1955).

Garden symphilids (*Scutigerella immaculata* (Newp.)) are present in the soil in most greenhouses, where they damage the root systems and stunt newly set plants. These pests were formerly destroyed only by steam sterilization or by fumigation of the soil with carbon disulfide between crops. Now it is possible to destroy the symphilids in the soil and protect the growing crop by watering in surface applications of lindane dust or wettable-powder spray. Many florists make annual applications of lindane to the ground beds of roses and some other crops to prevent the buildup of symphilids (Thomas 1949).

### CONTROL ON GREENHOUSE VEGETABLES

Inadequate pest control was formerly a limiting factor in the production of greenhouse vegetables. Infestations of spider mites, whiteflies, aphids, and thrips limited tomato and cucumber crops to the late fall and winter seasons. The new pesticides have made it possible to grow these crops also in the early fall and in the spring. Routine applications of parathion aerosol early in the growing period have eliminated most of the pests attacking greenhouse vegetables. Special treatments with malathion or TEPP aerosol when the crops were near maturity have given the desired pest control without undesirable residues on the harvested crops.

Other special treatments include DDT dust to destroy corn earworms and armyworms in fall crops of tomatoes and lindane applied to the soil for control of garden symphilids (Neiswander 1954, Porte and Smith 1955).



## SPIDER MITES RESISTANT TO CHEMICALS

The greatest threat to the continued control of greenhouse pests is the development of resistance, as exemplified by the two-spotted spider mite in certain commercial rose ranges. The resistance of this mite to parathion, first recognized in 1947 (Smith and Fulton 1949) in a few greenhouses of many examined, has since become widespread through plant sales to other establishments. In many greenhouses the level of resistance to parathion and a few other chemicals apparently became stabilized, and since 1951 commercial control has been obtained by use of sprays containing Aramite plus schradan. However, in a few ranges this combination treatment soon failed to give control, because surviving mites apparently mutated to new levels of resistance, since resistance is inherited by progeny in succeeding generations (Taylor and Smith 1956).

In certain greenhouses colonies of mites are now present that resist all miticides that have been tested against them. To avoid economic loss due to uncontrolled mite damage, rose growers are turning to chrysanthemums or other quick-maturing crops that can be harvested and remnants destroyed with any mites present before a new crop is planted. By replacing rose with mite-free plants the growers are successfully practicing cultural rather than chemical control to meet the resistance problem.

Another cultural practice that is being widely adopted is the reduction in greenhouse temperatures either by atomization of water under high pressure to give a fine mist or fog or by drawing in air with large fans through wet pads of excelsior. By this means the greenhouse temperatures can be reduced 15° to 20°F. and the quantity and quality of roses and other flowers greatly increased during the summer. It is too early to evaluate the effect of this practice on greenhouse pests, but flower thrips are not eliminated. Although the ventilators are kept closed the thrips pass through the excelsior pads. Spider mites develop more slowly at the lower temperatures, and preliminary tests indicate that they are more responsive to chemical control.

The cost of operating a greenhouse in the production of most crops has about doubled during the last decade, but the cost of pest control has remained about the same. Except for the few cases of highly resistant mites, greenhouse operators have benefited by the increase in quantity and quality of crops resulting from modern pest control (Smith, Brierley, and Lumsden 1948).

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# Narcissus Bulb Treatments at Planting-Time to Control the Narcissus Bulb Fly, *Lampetia equestris* (Fab.) (Diptera: Syrphidae)

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## ABSTRACT

Excellent control of the narcissus bulb fly was obtained in British Columbia by either spraying or dusting King Alfred narcissus bulbs with aldrin, dieldrin, chlordane, or heptachlor in the open furrow at planting time. Applications at this time of dusts of chlordane at 10, aldrin at 5, heptachlor at 5, or dieldrin at 3 lb. actual toxicant per acre (for rows planted three feet apart) gave almost complete protection to bulbs left in the field for three years. Sprays containing emulsifiable concentrates of chlordane at 5, aldrin at 2, heptachlor at 2, or dieldrin at 1 lb. toxicant per 100 gal. per acre were effective for at least two years. Soaking the bulbs for 10 minutes in cold water containing emulsifiable concentrates of aldrin at 5, chlordane at 5, dieldrin at 3, or heptachlor at 3 lb. toxicant per 100 gal. gave practically complete protection for three years. Treatments that did not prevent infestation during the season after planting were dusts of lindane at 1 or DDT at 5 lb. and granular (30-60 mesh) chlordane at 5 or heptachlor at 3 lb. toxicant per acre applied to bulbs in the open furrow, and a soak treatment of lindane at 0.5 lb. per 100 gal. of water. Lindane was the only insecticide tested that appeared to reduce root growth and the length of flower-stem.

## DISCUSSION

FLOYD F. SMITH. Do the larvae enter the neck area of the narcissus bulbs?

H. ANDISON. The larvae almost always enter the root ring area of the basal plate of the bulb. On rare occasions they have been observed to tunnel or mine the outer epidermal layers of the bulb before they enter the basal plate.



# The Biology and Control of *Macropsis fuscula* Zett., the Vector of the Rubus Stunt Virus

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## ABSTRACT

*Rubus stunt*, a virus disease of raspberries, has become increasingly prevalent in the Netherlands, particularly in the raspberry production area near Breda. The disease also occurs on wild blackberries; however, it has not yet been found in the Netherlands on cultivated blackberries (*Rubus procerus*).

Transmission experiments showed that the leafhopper *Macropsis fuscula* Zett. is the vector of the virus.

The biology and control of this leafhopper—which also occurs in Western Canada (Vancouver Island)—is discussed. Relationships between the virus and the vector are mentioned. The *Macropsis* fauna of the wild blackberry, being a possible source of virus infection for cultivated raspberries and blackberries, has been studied and is discussed.

## INTRODUCTION

In 1950 Prentice published a paper on the Rubus stunt disease, a dwarfing disease of loganberry, blackberry, raspberry and other cultivated species and hybrids of *Rubus*. The disease is caused by a virus, the Rubus stunt virus. It was first recorded in England by Wormald and Harris (1932). The disease later on became increasingly prevalent in loganberries, Phenomenalberry, blackberries and raspberries in Southern England; it probably affects all *Rubus* species. Infection results in the production of numerous weak, short and thin canes, which give the plant a bushy appearance; later the flowers often show typical proliferations, the floral parts becoming foliar. The incubation period of the symptoms in the field is about one year or even longer (Prentice, 1950; van der Meer, 1954).

Since 1954 the disease also has become increasingly prevalent in the Netherlands on raspberries in the production area near Breda, where it seriously interferes with their commercial cultivation. The disease has been known in that area for about 30 years; since 1945, however, there has been a sudden and rapid increase. Investigations on the spread and control were started in the Netherlands in 1950 by de Fluiter and van der Meer.

## RESULTS

Field observations soon indicated that the disease was vector-borne; they also showed that the disease in the Netherlands is common on wild blackberries (de Fluiter and Thung, 1951). In the many experiments carried out with the rubus aphids *Amphorophora rubi* Kalt. and *Aphis idaei* v. d. Goot no transmission of the virus was obtained. The leafhopper, *Macropsis fuscula* Zett., however, appeared to be a vector of the virus (de Fluiter and van der Meer, 1953). Symptoms of Rubus stunt first appeared in June 1953 in a series of experiments, started in July 1952, in which *M. fuscula* was tested as a vector of the virus. The incubation period of the disease on the test plants was in full agreement with the incubation period of the symptoms in the field. In the heavily infested area near Breda the first symptoms of the disease in plantations, planted with healthy plants, appear in late summer or in the autumn of the second year, viz., after the first crop is picked.

*Macropsis fuscula* Zett., as far as we know, lives only on *Rubus* species. According to literature the insect occurs in Italy, France, Belgium, the Netherlands, Germany, England, Denmark, Norway, Sweden, Finland, and northern Russia. Recently it has been introduced into Vancouver Island, Canada, where it is a pest of loganberry (Andison, in litt.).

The biology of *Macropsis fuscula* Zett. in the Netherlands has been studied. The leafhopper passes the winter as an egg on wild and cultivated *Rubus* species. The eggs are laid in the bark of the young canes. Hatching of the nymphs starts in the first half of May in a warm spring or in the second half of May in a cold spring. In the latter case hatching still continues at the beginning of June. The young nymphs feed on the young shoots, the leaf-stalks and the veins of the leaves.

In 1953, when spring was warm, the first adult leafhoppers were already present in the plantations at the end of June; at the end of July most of the *Macropsis* leafhoppers caught were adults. In 1954, with a cold spring, development was somewhat slower (Table I). Adults are present in the plantations during July, August and September. In this period the eggs are laid in the young canes. In September the leafhopper population decreases and by the end of September and the beginning of October very few mature leafhoppers are found on the first-year plants which at that time still bear green leaves.

TABLE I

Date of collecting 1954	Number of leafhoppers (nymphs + adults)	% adults
June 28	323	0.0
July 5	712	2.1
July 12	208	2.8
July 19	609	25.8
July 26	372	45.1
Aug. 2	224	76.3
Aug. 9	812	94.3
Aug. 16	608	99.3
Aug. 23–October 15	692	100.0

The virus is spread by the adult leafhoppers from the old plantations to the new ones. As plantations planted with healthy plants show 50–70% diseased plants by the autumn of the second year, and as the incubation period of the virus is about one year, the adult leafhoppers must be very active in the summer months and able to cover long distances.

That Rubus stunt virus is spread in August and September was shown by the following field experiment, among others:

Seventeen series of 50 healthy, potted raspberry plants of the variety Radboud were grown from virus-free rootstocks on an isolated field at Chaam. Every two weeks from April 16, 1953, a series of 50 plants was taken to a raspberry plantation near Beek, heavily infested with Rubus stunt. The pots were dug into the soil and pots and plants remained in the plantation for two weeks, when they were replaced by the next series. The last series was put in the plantation on October 26. As the plants were removed from the infested field they were carried to an insect-free glasshouse at Breda, where they stayed until the end of the season, in November, 1953, when they were taken out of the pots and planted in the isolated field near Chaam. Three series of check plants were included in the experiment: (a) one series of 50 plants which stayed throughout the season in the isolated field at Chaam; (b) one series of 50 plants which stayed throughout the season in the heavily infested plantation near Beek; (c) one series of 50 plants which stayed throughout the season in the insect-free glasshouse at Breda.

In November, 1953, the plants of the check series b and c were also planted on the isolated field near Chaam.

On November 5, 1954, and on May 26, 1955, the plants of all series were examined for the development of virus symptoms with the following result: Rubus stunt-diseased plants were only present in the series which stayed during the whole season in the heavily infested plantation, and in those series which stayed in this plantation during the periods from July 30 to Aug. 14, Aug. 14 to Aug. 28, and Aug. 28 to Sept. 14. The percentage of diseased plants in these series was 50, 3, 5, and 7 respectively. The period of virus spread thus coincided with the presence of adult *Macropsis* leafhoppers in the plantations.

The relation between virus and vector is still under investigation. The results of the preliminary experiments indicate a rather long latent period and a persistence of the virus in the vector. Five or 10 adult leafhoppers reared on virus-infected raspberry plants were transferred to healthy plants and allowed to feed on them for one to 21 days. Results are given in Table II.



TABLE II

Number of leafhoppers per plant	Date of infection and transmission feeding period						
	28-7-54	29-7-54	3-8-54	4-8-54	5-8-54	26-8-54	27-8-54
	1 day	5 days	1 day	1 day	21 days	1 day	6 days
5	-	-	-	-	x	-	-
5	-	-	-	-	-	-	-
5	-	-	-	-	x	x	x
5	-	-	-	-	x	-	-
5	-	-	-	-	-	-	-
10	-	-	-	-	x	x	x
10	-	-	-	-	-	-	-
10	-	-	-	-	x	-	x
10	-	-	-	-	x	x	?
10	-	-	-	-	x	x	x
10	-	-	-	-	x	-	x
10	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
10	-	-	-	-	x	-	x

- healthy plant  
X Rubus stunt diseased plant (symptoms developed in the autumn of 1955)  
? doubtful plant.

CONTROL

Leafhopper control means vector control, which in turn means control of virus spread. Field and laboratory experiments showed that the leafhopper can be controlled by the following schedules:

(a) A winter spray (January or early February) of tar oil 6%, or DNC (Aanitro) 0.4%, or DNC (Trifocide) 0.25%, which kill the eggs (de Fluiter and v. d. Meer, 1955). The winter spray is a very important and effective control measure if applied by all growers. We organized a large winter spraying campaign in 1954-55 with the help of the Horticultural Advisory service: the results are now becoming apparent.

(b) A spring spray of parathion 0.1%<sup>1</sup>, malathion 0.2%<sup>2</sup>, or diazinon 0.1%<sup>2</sup>, at the end of May or in the first half of June, to kill the young nymphs. Two applications of any of these materials at the rates given will reduce the leafhopper population greatly in those cases where the grower omitted the winter spray (de Fluiter and van der Meer, 1956).

(c) Spraying the young, first-year plantations during the critical period of virus spread in August and September to kill the virus-infected adults, and so to prevent infection of the young plantations. It would be very advantageous if a grower could thus protect his first-year plantations, as he would be less at the mercy of neighbouring growers who did not apply winter sprays. With this in mind we started control experiments in 1953, before we knew that *M. fuscus* was the vector of the virus.

Experiment 1. Every two weeks from May 15 to October 15, triplicated plots were sprayed with parathion 0.1%, Systox 0.1%, TEP 0.08%, or DDT 0.3%. A much smaller number of diseased plants appeared in the plots that received parathion or Systox (Table III).

Experiment 2. Systox at 0.1% was applied to triplicated plots every two weeks for varying periods beginning on April 15. Results (Table IV) showed that only those applications after August 1 had reduced the extent of disease. This is in agreement with our knowledge of the biology of the vector, *M. fuscus*.

MACROPSIS SPECIES ON RUBUS

We have already mentioned that, in the Netherlands, Rubus stunt is also a common disease of wild blackberries. Is the wild blackberry a source of virus for the cultivated

<sup>1</sup>Only allowed to be applied until 3 weeks before picking.  
<sup>2</sup>Allowed to be applied until 10 days before picking.

TABLE III

Insecticide	Diseased plants expressed as % of diseased plants in the check plots (= 100%).
Check	100
Parathion, 0.1%	22
Systox, 0.1%	30
TEP, 0.08%	55
DDT em., 0.3%	96.6

TABLE IV

Sprayed with Systox 0.1% once every two weeks in the period:	Average % Rubus stunt diseased plants
April 15–June 1	43.4
April 15–July 1	44.9
April 15–Aug. 1	37.5
April 15–Sept. 1	15.3
April 15–Oct. 1	16.1
April 15–Nov. 1	21.8
Check plots	34.4

raspberry (*Rubus idaeus*) and the cultivated blackberry (*R. procerus*)? Sampling in the field showed that in the south-western part of the Netherlands, in the province of Zeeland, *Macropsis scotti* Edw. is common on cultivated blackberries and certain species of wild blackberries; so far it never has been found on raspberries. Laboratory experiments showed that *M. scotti* cannot complete development on raspberries; nymphs collected on *R. procerus* and transferred to raspberries soon died. In laboratory experiments carried out by Mr. Pieper, adults of this species always preferred *R. procerus* to *R. idaeus*.

*M. fuscula* Zett. is very common on cultivated raspberries in the Breda area, where it was also collected from certain species of wild blackberry, viz., *Rubus gratus* and *R. nensis*. Laboratory experiments showed that nymphs of *M. fuscula*, hatched from eggs laid on raspberries, could develop into adults if reared on the wild blackberries *R. caesius*, *R. macrophyllum*, and *R. silvaticus*.

On *R. caesius* we also found *Macropsis* leafhoppers which differed slightly from *M. fuscula* Zett. In laboratory experiments the nymphs of this "species" developed into adults if reared on *R. caesius*, *R. macrophyllum*, and *R. silvaticus*; on *R. idaeus*, however, they soon died, which is in accordance with the results of experiments made in previous years.

Summarising, we can say that in the Netherlands *Macropsis scotti* Edw. only occurs on *Rubus procerus* (a cultivated blackberry) and on certain species of wild blackberry. If *M. scotti* Edw. is also a vector of the Rubus stunt virus—a point which is now under investigation—it may be able to transmit the virus from the wild blackberry to the cultivated blackberry (*R. procerus*).

*M. fuscula* Zett. is a vector of the Rubus stunt virus in raspberries. The fact that the species also can breed and mature on several species of wild blackberries indicates that the wild blackberry can act as a source of virus infection for cultivated raspberries.

The status of the *Macropsis* from *Rubus caesius* is still very doubtful. We are now studying its biology, life cycle and host preferences in order to investigate the part it plays in the spread of virus from wild blackberries to cultivated raspberries, if it is a species not identical with *M. fuscula* Zett.

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### DISCUSSION

A. HARTZELL. Did you find any evidence of another host in which the symptoms were masked?

H. J. DE FLUITER. In the field *M. fuscula* has been found only on *Rubus* spp. In laboratory experiments, however, we transferred *M. fuscula* from diseased raspberries to healthy strawberries. The next year some of these plants showed typical symptoms (a dwarfing and slightly proliferated flowers). The virus could not be transmitted from the infected strawberry plants to healthy ones by the strawberry aphid, *Pentatrichopus fragaefolii*.





# Acaricidal Properties of 2,4,5,4'-Tetrachloro-Diphenyl Sulphone (Tedion)<sup>1</sup>

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## ABSTRACT

2,4,5,4'-Tetrachloro-diphenyl sulphone (Tedion) has a specific action on immature stages of spider mites on many crops. It kills nymphs, larvae, and deposited eggs, and also affects the eggs developing in the ovary. The ovicidal and larvicidal action of Tedion compares favourably with those of 4-chlorophenyl 4-chlorobenzene sulphonate (CPCBS), 4-chlorophenylbenzene sulphonate (CPBS) and 4-chlorophenyl-4-chlorobenzyl sulphide (Chlorbenside). Tedion and CPBS do not significantly influence the longevity of females of *Tetranychus urticae* Koch. Tedion does not affect the fecundity of the females but CPBS causes a considerable decrease in the number of the eggs laid per female per day.

Treatment of bean plants with Tedion, CPBS or Chlorbenside affects the viability of the eggs of *Tetranychus urticae* developing in the ovary. The females produce sterile eggs for several days after contact with plants treated with Tedion. This effect is far less pronounced with CPBS and Chlorbenside.

In another experiment the upper surface of the leaf only was treated with Tedion, whereas the mites were placed on the under side. The females transferred after three days to untreated plants produced sterile eggs for several days. The influence of CPBS under these conditions is far less, and Chlorbenside shows no appreciable effect.

## INTRODUCTION

For several years acaricides with mainly ovicidal and larvicidal activity have been known, e.g., 4-chlorophenyl-4-chlorobenzene sulphonate (CPCBS), and 4-chlorophenylbenzene sulphonate (CPBS). Another two sulphur-containing compounds with comparable mode of action have appeared recently, viz., 4-chlorophenyl-4-chlorobenzyl sulphide (Chlorbenside) and 2,4,5,4'-tetrachloro-diphenyl sulphone (Tedion).

All these compounds are virtually non-toxic to the adult stages of the glasshouse red spider *Tetranychus urticae* Koch; nevertheless they are very useful in the control of plant-infesting mites. In two previous papers (Meltzer, 1955a, 1955b) experiments with acaricidal and ovicidal compounds have been described. The value of Tedion for the control of Tetranychid mites in the field has been proved by several small and large scale trials in various countries. Among the crops on which favourable results have been obtained in practice are fruits and hops, cucumbers, carnations, roses, beans, cotton, etc. The field experiences have been reported by Flik (1956).

This paper deals mainly with the influence of Tedion on the adult females and on the developing eggs in the ovary of *Tetranychus urticae*.

## EXPERIMENTS

### OVICIDAL EFFECTS OF CPCBS, CPBS, CHLORBENSIDE, AND TEDION

The ovicidal tests were divided in two series. In one series bean plants bearing eggs were dipped into emulsions of the compounds under consideration. In the other series non-infested plants were dipped, and after the plants had been dried they were infested with adult females in order to obtain eggs on the dry deposit. Fig. 1 shows the plexiglass chambers used for confining the mites on the leaf. After two days the chambers and the adult mites were removed. Records were taken 8 days after removal of the females.

The mortality percentages were computed with Abbott's formula,  $\frac{a-b}{a} \times 100\%$ , in which 'a' represents the number of survivors in an untreated control, and 'b' the survivors in the treatment concerned. Control mortality in these tests was very low, as a rule less than 3 per cent, rarely more than 5 per cent and never exceeding 10 per cent.

Communication No. 41 of the Agrobiological Laboratory "Boekesteijn", 's-Graveland, The Netherlands.

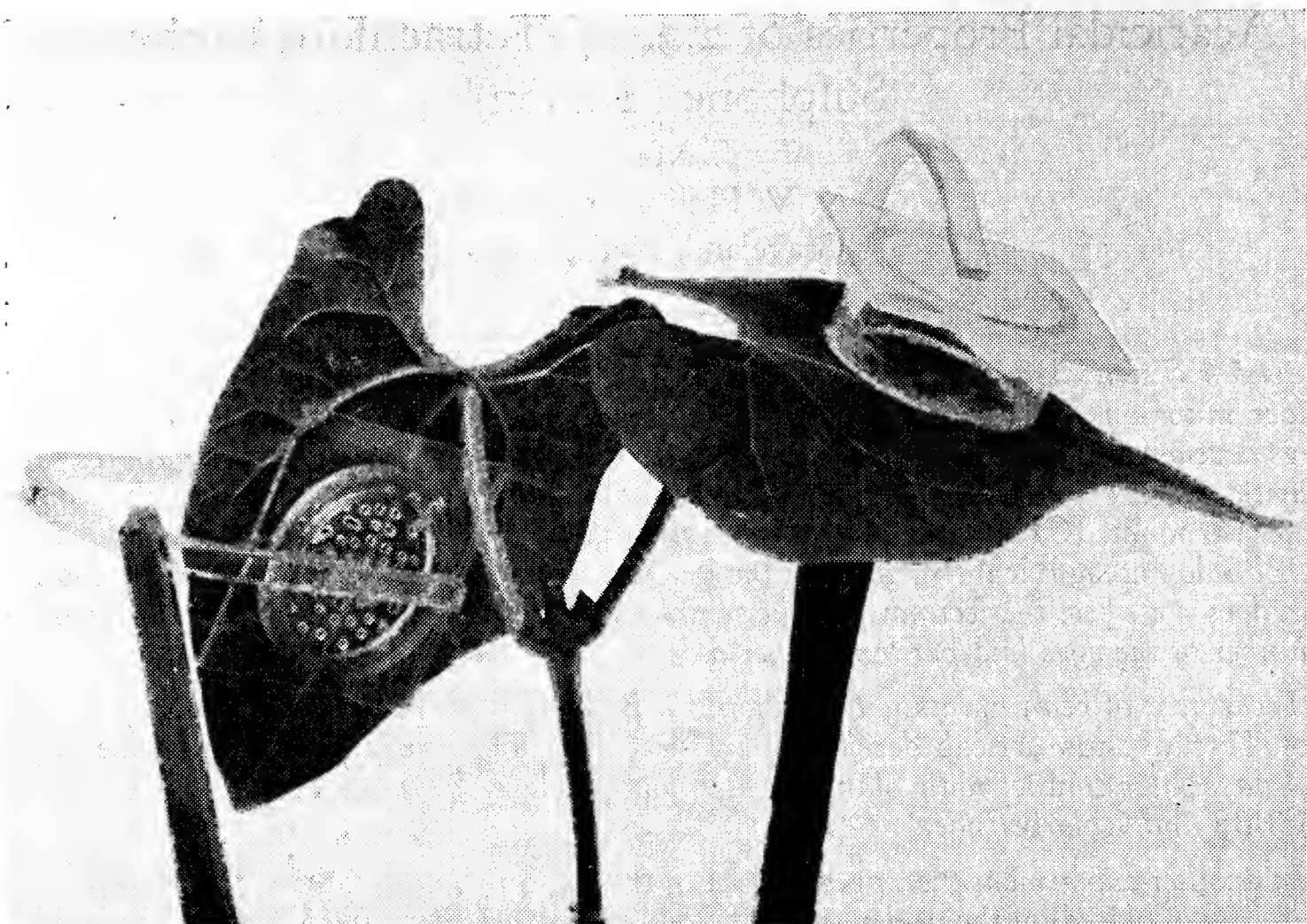


Fig. 1. Plexiglass chamber used to confine mites on leaves.

As can be seen in Table I, mortalities given by the two methods of application of a material usually differed, the extent of the difference varying with the material.

TABLE I. Mortality of Eggs and Larvae of *Tetranychus urticae* on Bean Leaves Dipped into Emulsions or Laid on Dry Deposits of some Ovicidal Compounds.

Compound	Concentr. active material, p.p.m.	Number of expts.	Dipped eggs		Eggs laid on dry deposit	
			% kill of eggs	% total kill*	% kill of eggs	% total kill*
CPCBS	100	8	79	100	71	89
	30	7	54	100	28	45
	10	5	26	48	9	20
	3	2	1	2	8	28
CPBS	300	16	75	100	93	100
	100	19	55	98	69	88
	30	6	39	73	12	63
	10	3	1	43	1	8
Chlorbenside	300	1	100	100	100	100
	100	5	60	98	63	79
	30	5	39	85	62	65
	10	5	17	44	49	56
	3	4	17	42	23	34
Tedion	100	30	98	100	100	100
	30	40	90	100	99	100
	10	32	81	99	98	100
	3	25	40	96	95	100
	1	12	8	91	68	86
	0.3	7	3	52	43	57

\*Kill of larvae and nymphs included.

#### EFFECT OF TEDION AND CPBS ON THE LONGEVITY OF FEMALES

In order to determine the longevity of the adults under laboratory conditions 10 bean leaves with 20 females each were used in a blank experiment. The adults were transferred

daily to fresh plants to provide food of good quality. The mortality curve for 15 days is shown in Fig. 2.

In the experiment with Tedion and CPBS the females were transferred each day to fresh plants treated by dipping in emulsions of the acaricides. A control series, never exposed to the compounds, was also transferred daily to untreated leaves.

The mortalities of the mites on leaves treated with Tedion at 30 and 300 p.p.m. were initially lower than in the control (Fig. 2), but as mortality among those exposed to 100 p.p.m. was similar to that of the control, Tedion probably has no significant effect on longevity of adults.

The mortalities on the plants treated with CPBS (Fig. 2) were also lower during the first three days than in the control, but later only the mortality from the lowest concentration remained lower. The mortality from 200 and 300 p.p.m. after the first week tended to be greater than in the control but the differences were not statistically significant.

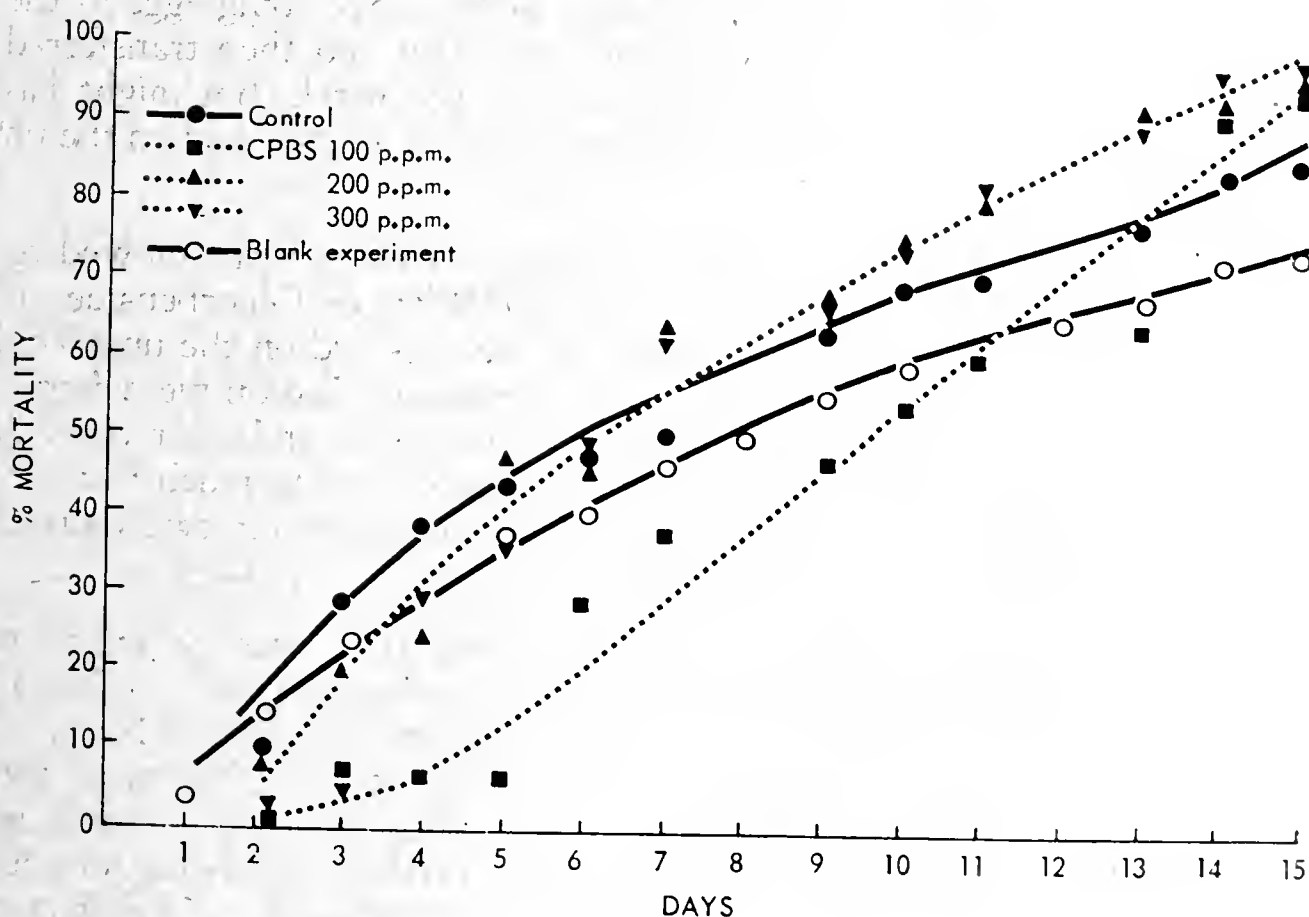
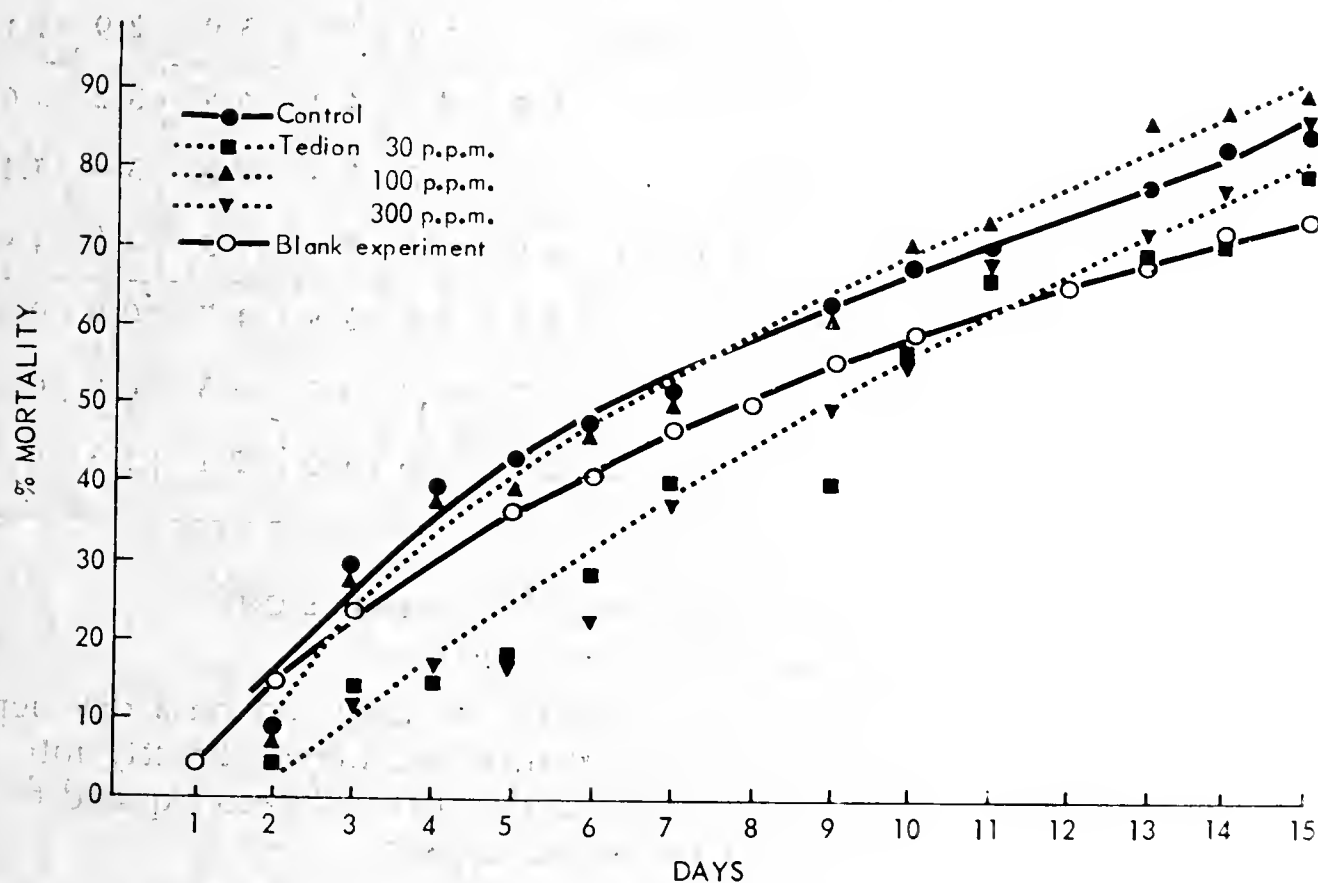


Fig. 2. Mortalities of adult females of *Tetranychus urticae* on leaves treated with Tedion (above) and CPBS (below).

EFFECT OF TEDION AND CPBS ON THE FECUNDITY OF FEMALES

In the preceding experiments on longevity the daily egg production was recorded. In the blank experiment, the production averaged 4.67—0.2 eggs per female per day during fifteen days. Fecundity of females on plants treated with Tedion did not differ significantly from that of the control (Table II).

The fecundity of females on plants treated with CPBS, however, was much lower; the differences between all concentration of CPBS and the control were highly significant.

TABLE II. Average Daily Egg Production by Females of *Tetranychus urticae* on Bean Leaves Treated with Acaricides. Initial Age of Females 0–3 Days.

Treatment, material in p.p.m.	Initial number of females	Mean egg production per female at day given												Average
		1/2	3	4	5	6	7	8/9	10	11	12/13	14	15	
Control	40	6.4	3.7	4.3	7.4	6.5	4.8	5.0	5.6	4.5	3.9	2.9	4.1	5.1 ± 0.
Tedion 30	35	7.3	5.0	4.9	4.6	7.7	3.6	5.5	4.8	4.2	6.5	3.2	3.6	5.1 ± 0.
100	41	5.6	4.6	5.1	5.0	9.5	4.0	3.6	11.3	5.6	4.6	3.8	5.1	5.7 ± 0.
300	37	8.7	5.3	5.2	7.7	6.9	4.0	4.6	6.1	3.9	5.5	4.4	4.9	5.8 ± 0.
CPBS 100	36	8.4	3.5	3.8	1.2	3.5	1.0	3.8	4.0	3.8	1.7	0.9	0.0	3.4 ± 0.
200	42	1.0	2.6	0.3	0.0	1.8	1.2	3.0	0.8	4.5	4.3	0.0	0.0	1.6 ± 0.
300	42	1.5	2.9	1.8	0.5	0.8	0.2	2.0	0.6	1.6	2.1	3.3	0.0	1.4 ± 0.

EFFECT OF TEDION, CPBS, AND CHLORBENSIDE ON  
DEVELOPING EGGS IN THE OVARY

In the experiments on eggs mortality (Table I), the eggs laid on a dry deposit of Tedion showed a considerably higher mortality than those dipped directly into Tedion emulsions. It was first supposed that the eggs of females that had been exposed to Tedion were more susceptible than those laid by females not exposed.

To investigate the possible influence of Tedion on the developing eggs in the ovary, females were placed on Tedion-treated plants for three days and then transferred to untreated plants. The eggs laid on the treated leaves did not hatch, but might have been killed directly by the deposit of Tedion; however, those subsequently laid on the untreated leaves also did not hatch.

Further experiments were then carried out in which females were allowed to remain for three days on plants bearing deposits of Tedion, CPBS, or Chlorbenside, and then transferred daily to fresh, untreated plants. Most of the eggs laid on the untreated plants during the first five days by the females previously exposed to Tedion were sterile (Table III). After this period the proportion of viable eggs increased, and after eight days the effect of Tedion was practically lost. The other two acaricides had much less effect, that of CPBS persisting for only four days and that of Chlorbenside for two days after the females had been transferred to untreated plants.

In another experiment, only the upper surface of the leaves was treated by brushing them with acetone solutions of the compounds. Females were allowed to feed for three days on the under side of the treated leaves and then transferred daily to fresh, untreated plants. The mortality of eggs laid on the treated leaves was comparable for all three compounds (Table IV). After the females were transferred to untreated leaves, however, only Tedion showed a prolonged effect on the viability of the eggs, lasting for about eight days at the 1000 p.p.m. level and about two to four days at 100 p.p.m. The effect of CPBS at 1000 p.p.m. lasted two to four days and at 100 p.p.m. one to two days. Chlorbenside showed no appreciable effect on the developing eggs at these concentrations.



TABLE III. Mortality of Eggs of *Tetranychus urticae* from Females Confined for Three Days on Leaves Treated with Acaricides and then Transferred Daily to Untreated Leaves.

Treatment, material in p.p.m.	Percentage kill of eggs (E) and total kill (T)*																
	On treated leaves	On untreated leaves on day given															
		1		2		4		5		6		8		9			
		E	T	E	T	E	T	E	T	E	T	E	T	E	T		
S 1000	95	100	54	74	15	55	11	20									
	90	100	14	51	18	60	11	19	3	30	0	0	0	0			
rbenside 1000	100	100	36	40	8	12	7	14									
	100	99	100	52	66	2	16	0	1								
	30	100	100	57	64	4	11	3	3								
on 1000	100	100	100	100	100	100	100	100	100	100	91	96	49	55	9	19	
	100	100	100	100	100	100	100	100	100	86	90	62	65	16	21	3	11
	30	100	100	100	100	100	100	96	98								

\* If larvae and nymphs included.

TABLE IV. Mortality of Eggs of *Tetranychus urticae* Laid by Females Confined for Three Days on the Under side of Leaves Treated only on the Upper Side with Acaricides, and then Transferred Daily to Untreated Leaves.

Treatment, material in p.p.m.	Percentage kill of eggs (E) and total kill (T)*													
	On treated leaves	On untreated leaves on day given												
		1		2		4		5		6		8		9
	E	T	E	T	E	T	E	T	E	T	E	T	E	T
1000	100	100	83	100	19	93	6	75	0	0	3	3	0	12
100	88	100	38	91	10	56	3	33	0	23	10	34	2	2
opside 1000	100	100	14	27	0	7	3	7	0	0	0	3	0	0
100	96	100	12	30	0	0	19	19	0	0	0	0	2	2
n 1000	92	100	100	100	100	100	88	90	94	100	100	100	100	100
100	91	100	100	100	85	89	86	91	6	11	9	9	0	8
													19	19
													3	11

\* If larvae and nymphs included.

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# Scope for Using Vegetable Oils as Insecticides

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## ABSTRACT

Vegetable oils have been used as insecticides, synergists, and carriers. The insecticidal potentialities of vegetable oils, however, have not been very great due to many factors—high concentrations required, uneconomical and phytotoxic effects. Soap emulsions of oils of castor, custard apple seed, groundnut, tobacco, sesamum, linseed, safflower, karanj and neem have been tested under laboratory conditions on safflower aphid, *Achoea janata*, *Euproctis guttata*, *Porthesia scintillans* and *Urentius echinus* and the eggs of the moths. Most emulsions were effective against the early instars of caterpillars and custard apple seed oil gave the best results. Aphids and lace wing bugs are affected at 5% level. The eggs of *Achoea* were affected more adversely than the eggs of *Euproctis* and *Porthesia*.

Vegetable oils were found phytotoxic to castor, maize, and paddy, even at 0.5% concentration. Castor leaves produce necrotic spots and veins become blackened. Maize leaves develop specks in lines which can be confused with virus symptoms. Paddy leaves show severe necrotic spots, some times as streaks. The affected areas do not recover. Intensity of symptoms increases with increasing concentrations. High temperatures add to the severity of the disease. Groundnut and safflower leaves were not affected.

The most important uses of vegetable oils have been industrial, medicinal, and domestic; however, some vegetable oils had been in use as insecticides for a long time, mostly in the old world. In the present study the following oils have been tested against certain pests of castor and other crops and a study has been made of their phytotoxic effects on castor, groundnut, paddy, and maize:—Castor, *Ricinus communis*; Custard apple, *Anona squamosa*; Groundnut, *Arachis hypogea*; Tobacco, *Nicotiana tabacum*; Sesamum, *Sesamum indicum*; Linseed, *Linum usitatissimum*; Safflower, *Carthamus tinctorius*; Karanj, *Pongamia globra*; Neem, *Azadirachta indica*; and Mustard, *Brassica juncea*.

Soap emulsions of these oils were prepared in concentrations ranging from 1 to 10 per cent. Emulsions were made by slowly adding required quantities of oil to warm soap solution, which was prepared by dissolving one ounce of laundry soap in one gallon of warm water. As crusts form on standing, fresh emulsions were always used.

Laboratory trials were conducted with various vegetable oils against safflower aphid, *Macrosiphum* sp., castor semilooper, *Achoea janata*, brinjal lace wing bug, *Urentius echinus* (Tingidae), *Porthesia scintillans* and *Euproctis guttata*. Preliminary field trials were made against castor semilooper. Laboratory tests were made with the help of Potter's tower.

*Macrosiphum* sp.—One, three, and five per cent soap emulsions of mustard, custard apple seed, tobacco seed, and neem oils were tested against the winged, apterous, and nymphal stages of the aphid. All the concentrations of mustard and 5% custard apple seed oil gave 100% mortality 24 hours after treatment. Neem and tobacco oils at 1 to 5%, and custard apple seed oil at 1 and 3% gave kills ranging from 40 to 98%.

Castor semilooper, *Achoea janata*.—All five instars of castor semilooper were exposed to 1, 5, and 10% oil emulsions of custard apple seed, mustard, groundnut and Karanj. Within 24 hours after treatment 100% kill of first and second instar caterpillars was obtained with 10% custard apple seed oil, whereas 72 hours were required to obtain 90% kill of third and fourth instar caterpillars. The mortality of caterpillars ranged from 20 to 70% with other oils used, the final instars remaining almost unaffected. During the experiment it was noticed that caterpillars were feeding normally.

In a field trial against castor semilooper, 1, 5, and 10% emulsions of castor, mustard, groundnut, and sesamum were sprayed with a knapsack sprayer over an area of 1/40 acre for each treatment. Observations showed that 5 and 10% mustard and groundnut oils gave better kill than castor and sesamum. However, there was a severe burning of plants, hence further observations could not be continued. There was no kill and no phytotoxic effect where soap solution alone was sprayed.

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Brinjal lace wing bug, *Urentius echinus* (Tingidae).—One, three, five, seven, and ten percent oil emulsions of custard apple seed, mustard, groundnut, and neem were used in the laboratory against adults of brinjal lace wing bug, which sometime becomes a serious pest. Mortality of 90% was obtained with 10% custard apple seed, groundnut and neem oils 72 hours after treatment. Concentrations of seven and five percent closely follow ten percent in their effect. Concentrations lower than 5% are not of much value because of their low toxicity to insects.

*Porthesia scintillans*.—Soap emulsions of custard apple seed, mustard, sesamum, and neem oils were used against all instars. Concentrations of one, five, and ten percent were applied. Custard apple seed oil at 1 to 10% gave 100% kill of first and second instar caterpillars whereas 10% mustard and neem oils gave 95 and 90%, and 90 and 85% mortality of first and second instars respectively. The other oils were not very effective and the mortality did not exceed 75%.

*Euprotis guttata*.—Caterpillars were treated with one, five, and ten percent soap emulsions of custard apple seed oil. The mortality did not exceed more than 10% with any concentration.

Ovicidal Action of Vegetable Oils.—Concentrations of one, three, and five percent soap emulsions of custard apple seed, castor, mustard, neem, and groundnut oils were sprayed on the eggs of castor semilooper, *Achoea janata*, *Porthesia scintillans* and *Euprotis guttata*. The eggs of castor semilooper were affected chiefly with groundnut oil and the hatch never exceeded 25% whereas in control it was 75%. The eggs of *Euprotis* were influenced less than the eggs of *Porthesia* by custard apple seed, neem, and mustard oil emulsions.

Phytotoxicity of Vegetable Oils.—It is well known that mineral oils are phytotoxic to foliage of many plants even at low concentrations. Many authors have recorded the phytotoxic effects of vegetable oils on different crops. The present study is the result of efforts to find out the possibility of using vegetable oils as insecticides. Vegetable oils in soap solutions mentioned elsewhere have been used in these studies on castor, maize, paddy, wheat, safflower, groundnut, whitelilly, *Acalypha* and sunflower. Soap solution and water were used separately as checks. Symptoms:—General symptoms due to phytotoxic effects of vegetable oils can ordinarily be confused with the symptoms produced by some parasitic organisms. In many instances leaf spots are caused, causing local necrosis, sometimes small specks and long streaks are formed; veins become blackened, leaves curl upwards, and finally dry up. In cases where the plants are small the whole plant dries up and dies. All the sprayed portions assume an oily appearance. Symptoms become more prominent and appear sooner when days are bright, cloudy weather having a depressing effect.

Usually symptoms on different plants are similar with different vegetable oils, only varying slightly in intensity and degree of susceptibility. Concentrations of 1 to 10% of mustard, neem, Karanj, groundnut, castor, sesamum, linseed and custard apple were sprayed on castor to study the phytotoxic effects.

Castor plant:—The symptoms appear on leaves as small spots. The veins become blackened and the tips begin to dry. Symptoms appear fully one week after treatment. Severely affected leaves become scorched and curl upwards. The affected leaves become yellowish and fall off easily. The stems and leaf stalks, when sprayed, get spots and the bloom disappears. Small plants are killed with 10% concentration. Severity of symptoms increases with increasing concentrations (Fig. 1.).

Maize:—First symptoms appear as chlorotic spots. Later these develop into small spots and resemble those caused by *Physoderma*. The effect of mustard oil was very severe and all the leaves sprayed dried up.

Paddy:—Dark brown spots develop and later coalesce into streaks. Affected portions do not recover; however, entire leaves do not dry up.

Wheat:—Only slight discolouration was noticed on wheat leaves.

Safflower:—Where 10% emulsions were used symptoms of tip burning were noticed.



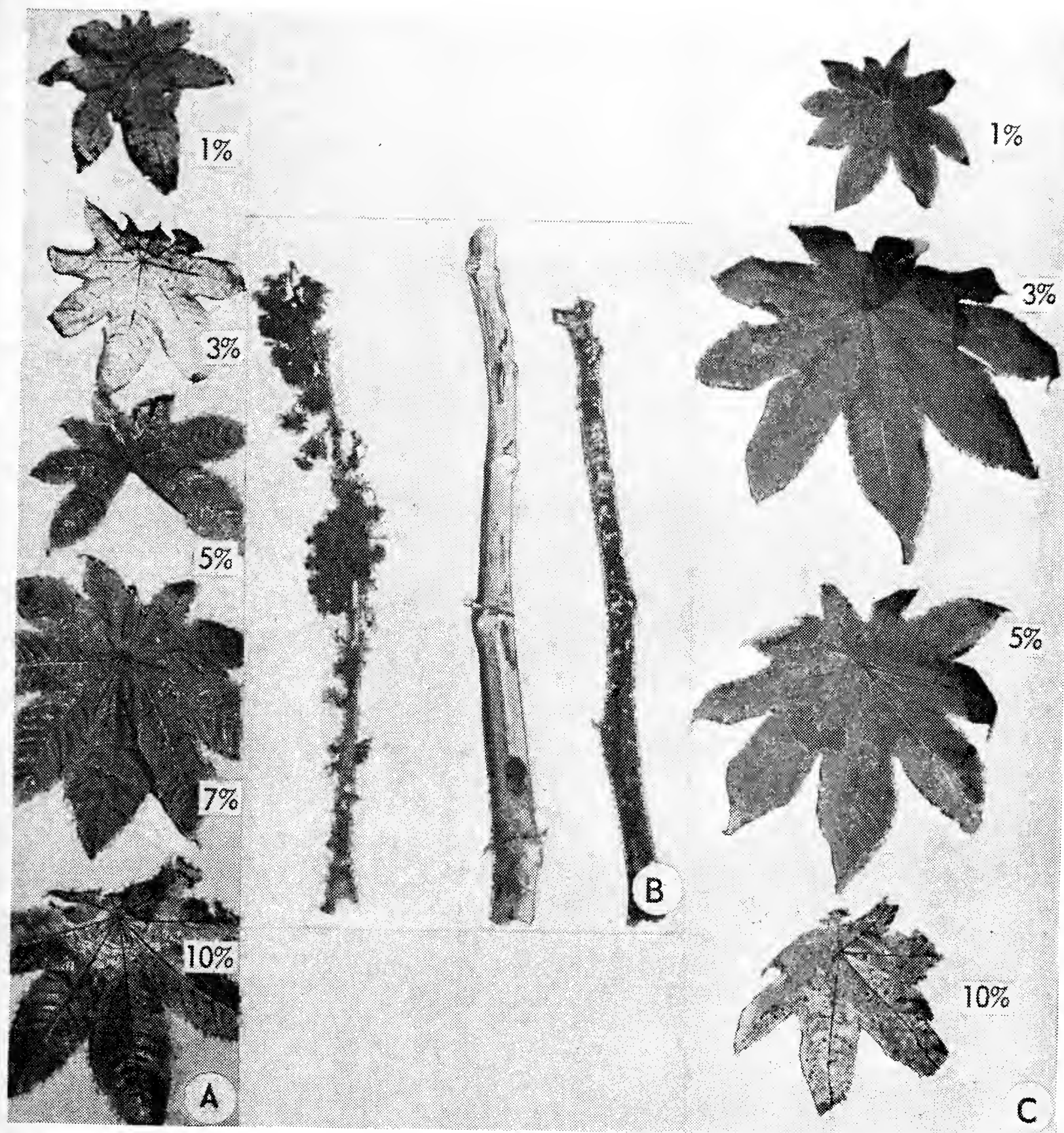


Fig. 1. Toxic effects of vegetable oils on castor. A, Castor oil on leaves. B, Castor oil on stalks and spikes. C, Mustard oil on castor leaves.

Sunflower:—Excepting mustard, no oils used had any effect on sunflower leaves. Small spots appeared with 5 and 10% mustard oil emulsion. All the oils tried had no effect on groundnut, *Acalypha*, and white lilly, *Crinum* sp.

The tests conducted showed that 0.5% to 10% oil emulsions caused symptoms on plants found susceptible. All oils except 1% neem, affected maize. All oils above 1% concentration showed symptoms on paddy. All oils had phytotoxic effects on castor, even at 0.5% concentration. There was no injury to any plant sprayed with soap solution and water.

The possibility of using vegetable oils as insecticides is very limited. The high concentrations required to kill the insects, high cost, and the large quantities of oils required per acre make their use uneconomical. The observations show that the phytotoxic effects of almost all oils tested make their use unsuitable on many crops. Moreover, vegetable oils as such are not very potent insecticides, except the custard apple seed oil which is highly toxic against quite a few insects. Vegetable oils, however, can be used as synergists, carriers, and stickers with other insecticides.



# Experimentos con Insecticidas Sistemicos en el Algodon Tanguis del Perú

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## ABSTRACT

*Systox 0.05% and metasystox 0.1% reduce the population of Empoasca sp. on cotton fields, but metasystox does it quicker and has a larger residual power.*

*No satisfactory control of Pinnaspis minor Mask (Coccidae) was obtained with metasystox 0.1%.*

*No influence of the time of application of metasystox against Leucothrips theobromae Priesn. was observed, but the dosage was demonstrated to have a real influence in the control of this Thysanoptera.*

*Instead of the different dosages (up to 1 lt./Ha.) and different forms of application (sprayed, injected, watering) only contradictory results against Pseudococcus citri were obtained with systox and metasystox on Tanguis cotton.*

*Systox controls Aphis gossypii during 4 to 5 weeks, metasystox during 6 weeks and more completely. OMPA makes one week unsatisfactory control. Primin acts by contact but not like a systemic insecticide. Sytam gave a regular control but only during 16 days. Metasystox when injected into the soil did not work. Thimet (87 kg. Ha. 50% activated carbon) applied into the soil gives excellent control. Compound 4739 (18 Kg. Ha.) shows systemic action.*

*Increasing dosages of metasystox gives a better control and longer residual effect against Aphis. Incompatibility of calcium arsenate with systox and metasystox was observed; also the systemic insecticide was less toxic than other organic insecticides.*

*The mite, Paratetranychus peruvianus, is controlled by metasystox 0.1% but takes 4 days for its destruction.*

Durante cinco años hemos visto, en el Perú, a los insecticidas sistémicos, especialmente Systox y Metasystox, fulminar insectos chupadores-picadores, al extremo de no permitir la vida de un solo ejemplar durante varias semanas en los algodones tratados y también los hemos visto demostrar una irritante ineficacia contra insectos del mismo grupo de los picadores-chupadores. Los resultados de esos cinco años de experimentación con este tipo de insecticidas, es lo que me propongo dar acá a grandes rasgos.

Trataremos, en primer lugar, de los ensayos realizados con Systox y Metasystox en nuestro afán de controlar a los insectos del género *Empoasca* (Fam. Jassidae), causantes de la anomalía del algodón conocido como "hoja crespada" y del ahilamiento de las plantas de la misma malvacea, con una severa pérdida de cosecha, por ausencia de ramas fruteras.

De los muchos ensayos de campo realizados sólo citaremos dos. En el primero se empleó el Systox al 0.05% en pulverización aérea. Los resultados que se ven en la Figura N° 1 indican que a los 6 días de la aplicación en las parcelas de Systox existían 19% de plantas con *Empoasca* mientras que en el testigo habían 47%. La población de plantas con *Empoasca* se mantiene así alrededor de 20% hasta 27 días después de la aplicación del Systox en que sube hasta ponerse por encima del testigo a los 33 días, para de ahí en adelante seguir una carrera prácticamente paralela con el testigo indicando así que ya no hay más acción del insecticida y que su poder residual, contra este insecto, no vá más allá de las cuatro semanas.

En el segundo ensayo en que intervinieron Systox al 0.015% y Metasystox al 0.03% se obtuvieron resultados similares como puede verse en la Figura N° 2, ya que tanto Systox como Metasystox controlaron a la *Empoasca*. Sin embargo permítaseme llamar vuestra atención hacia el hecho de que Metasystox ejerce una acción más violenta y más prolongada que Systox contra *Empoasca* ya que, a los 3 días, había bajado la población de plantas atacadas, desde 74%, hasta 12%, mientras que Systox sólo había descendido de 74% a 44%, en el mismo lapso de tiempo y mientras que en las parcelas que recibieron Metasystox el número de plantas atacadas por *Empoasca* sigue bajando y llega a cero a los 33, para de

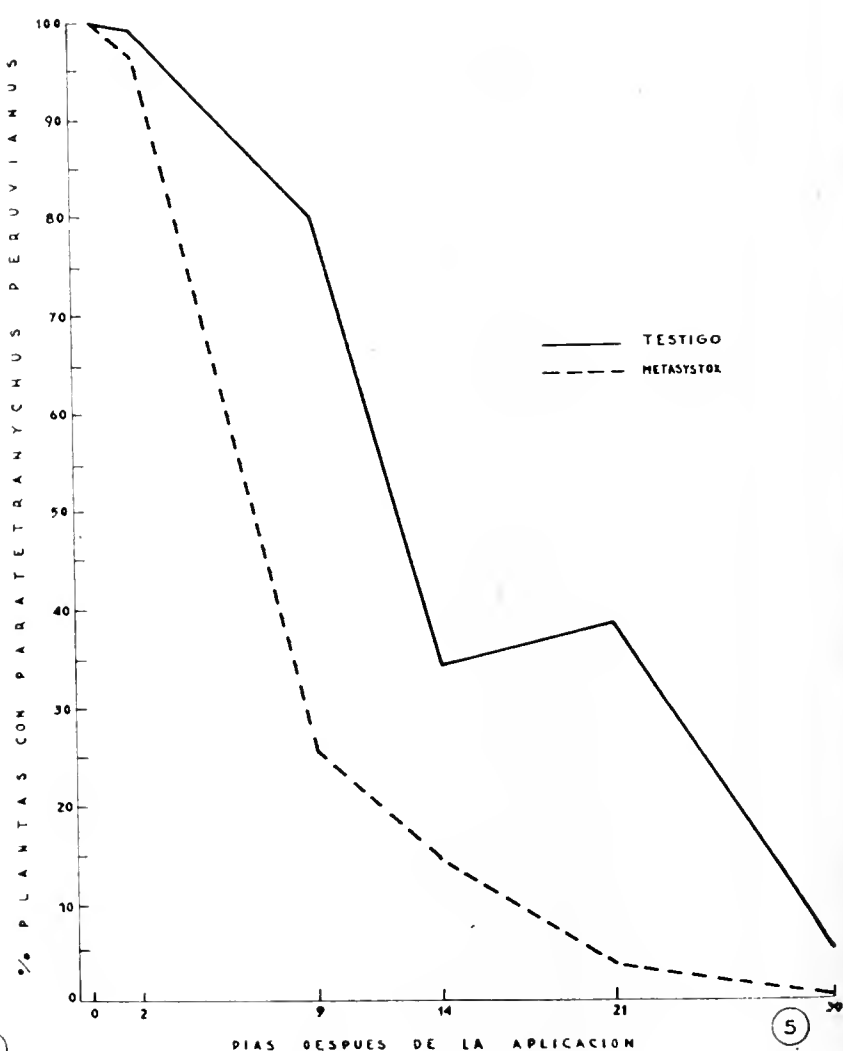
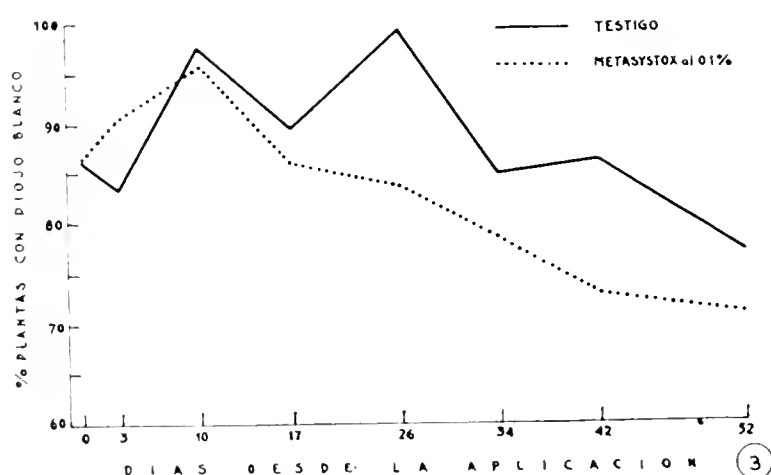
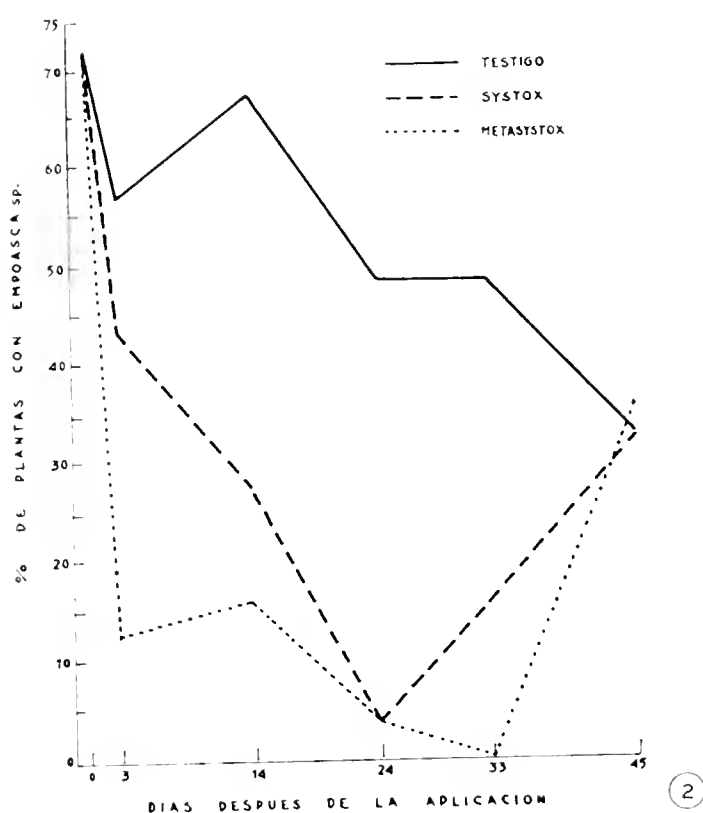
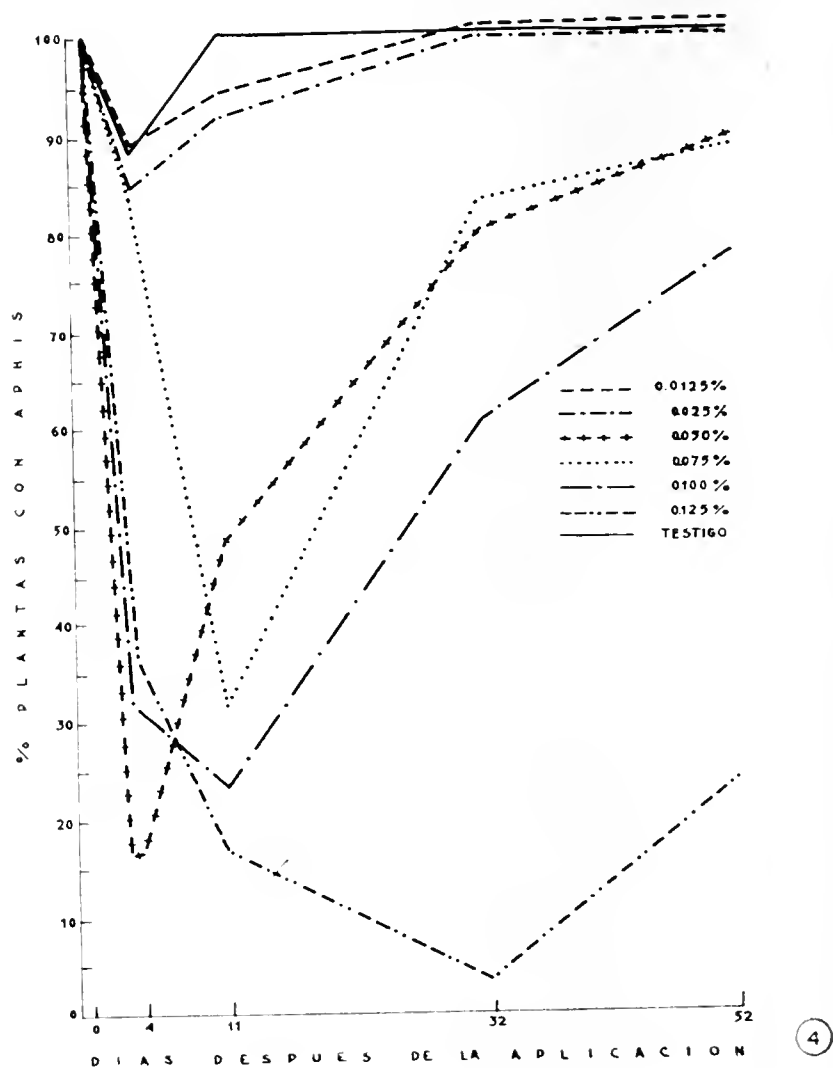
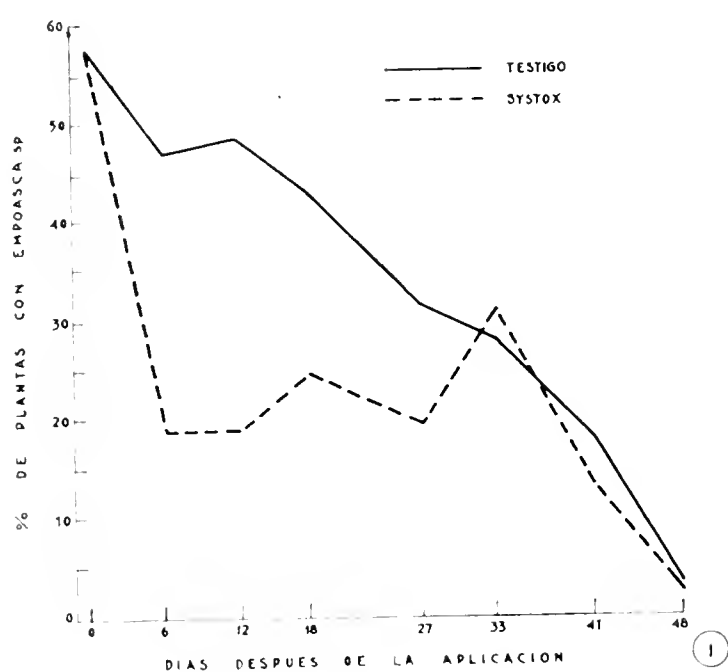


Fig. No. 1. Curva de gradación de *Empoasca* sp. en el experimento conducido en la Hda. de la Escuela Nacional de Agricultura (Lima, 1955).

Fig. No. 2. Curva de gradación de *Empoasca* sp. en el experimento conducido en la Hda. Huarabí Alto (Lima, 1954-55).

Fig. No. 3. Curva de gradación del Piojo Blanco, *Pinnaspis minor*, en el experimento llevado en la Hda. Macas (Lima, 1955).

Fig. No. 4. Gradación de *Aphis gossypii* en el ensayo sobre dosis de Metasystox conducido en la Hda. Trapiche (Lima, 1954).

Fig. No. 5. Gradación del ácaro *Paratetranychus peruvianus* en el experimento realizado en La Molina (Lima, 1956).



ahí comenzar a subir, en el caso del Systox baja sólo hasta los 24 días, sin llegar a cero, y luego sube. La línea del testigo, siempre por encima de Systox y Metasystox demuestra la buena acción de estos dos productos sistémicos.

Contra *Pinnaspis* (*Hemichionaspis*) *minor* Mask. (Fam. Coccidae) que ataca al algodónero causándole daños, especialmente después del segundo corte, fué ensayado en Perú el Metasystox al 0.1% sin resultados satisfactorios como puede verse en el gráfico de la Figura N° 3, si bien es cierto que dejó entrever una ligera acción insecticida contra esta queresa del algodón Tangüis.

*Leucothrips theobromae* ataca también al algodón Tangüis en el Perú. Se presentó durante la última temporada en dos de los ensayos que se estaban llevado a cabo.

Uno de los ensayos se proponía dar a conocer cual era la mejor época de aplicación del Metasystox (el insecticida sistémico de uso comercial más difundido hasta la campaña 1955-56 en el Perú), cuestión muy importante tratándose de un algodón de período vegetativo largo (7 a 13 meses) y de un país en el que los insecticidas son caros. El otro ensayo se proponía conocer cuál era la dosis más apropiada para su uso en el control de los diversos insectos picadores-chupadores del algodónero.

En el primer ensayo se hicieron cuatro aplicaciones de Metasystox al 0.1% con un intervalo de dos semanas entre cada una. En el segundo se aplicó Metasystox al 0.050%, al 0.100%, al 0.125% y al 0.150%.

Los resultados demostraron que el Metasystox al 0.1% no ejerce un control satisfactorio del *L. theobromae* cualquiera que sea la época de aplicación (gráfico de la Figura N° 6) pero que el grado de control del Thysanoptero aumenta a medida que sube la dosis como se vé claramente en el gráfico de la Figura N° 7.

Tanto Systox como Metasystox se han empleado en varios experimentos desde el año 1951 contra el *Pseudococcus citri* con resultados siempre negativos. Se han usado los insecticidas en dosis crecientes hasta llegar a proporciones tan altas como 1 litro por hectárea. Se han aplicado pulverizados a la parte aérea de la planta, inyectados al suelo con inyector ad-hoc o regados al suelo sea con el agua de riego o independientemente y siempre los resultados fueron negativos o dudosos.

Cuadros típicos de los muchos resultados insatisfactorios en el control de *P. citri* son los gráficos de las Figuras N°s 8 y 9. En el primero se ve que mientras en el testigo sin aplicación hubo una población promedio de 16% de hojas infestadas por *Pseudococcus* en las parcelas tratadas con 330, 500 y 700 cm<sup>3</sup> de Metasystox por hectárea en pulverización aérea hubieron 16, 14 y 18% de hojas infestadas por *P. citri*, respectivamente.

En el otro gráfico (Fig. 9) se trata de las poblaciones de hojas infestadas por *P. citri* en las parcelas en las que se inyectó 330, 500 y 700 cm<sup>3</sup> de Metasystox por Ha. valiéndose de un inyector de mano y dándole un golpe por mata a 20 cm de profundidad. Los resultados, como se ve, son 38%, 48% y 44%, respectivamente para las parcelas tratadas y 41% en el Testigo.

Así, en el Perú, hemos llegado a la conclusión, después de porfiar casi una docena de veces, de que ni Systox ni Metasystox controlan al *Pseudococcus citri* en el algodón Tangüis.

El *Aphis gossypii* también parasita a la planta del algodónero en el Perú ocasionando pérdidas que en los casos más severos alcanzan casi el 100% de la cosecha.

El uso de insecticidas arsenicales para el control de los gusanos de la hoja, *Anomis texana* y *Alabama argillacea* y de insecticidas orgánicos contra estos y otros insectos, en los últimos 10 años, han traído siempre asociada la aparición de una fuerte plaga de *Aphis gossypii*. Una de las más caras aspiraciones de los entomólogos en el Perú era conseguir un insecticida que fuera lo más específico posible contra el Aphis, lo más económico y que no destruyera el balance biológico ocasionando plagas secundarias; por eso a la llegada de los insecticidas sistémicos, se tuvieron grandes esperanzas para la solución de este problema y se realizaron, desde entonces hasta la fecha, numerosos ensayos de este tipo de productos contra el *Aphis gossypii* ó pulgón de la melaza de algodónero. Enumerar los resultados obtenidos en cada ensayo tomaría muchas veces el tiempo de que disponemos, por élló sólo tomaremos los casos más claros, como por ejemplo aquel de ensayo llevado en una hacienda cerca a Lima, en el que se empleó Systox a razón de 165 cm<sup>3</sup> por Ha. y Metasystox

a razón de 330 cm<sup>3</sup> por Ha. en comparación entre sí y con un testigo que sólo recibió 23 Kgs. de Azufre por Ha. y cuyos resultados los expresamos en el Cuadro I.

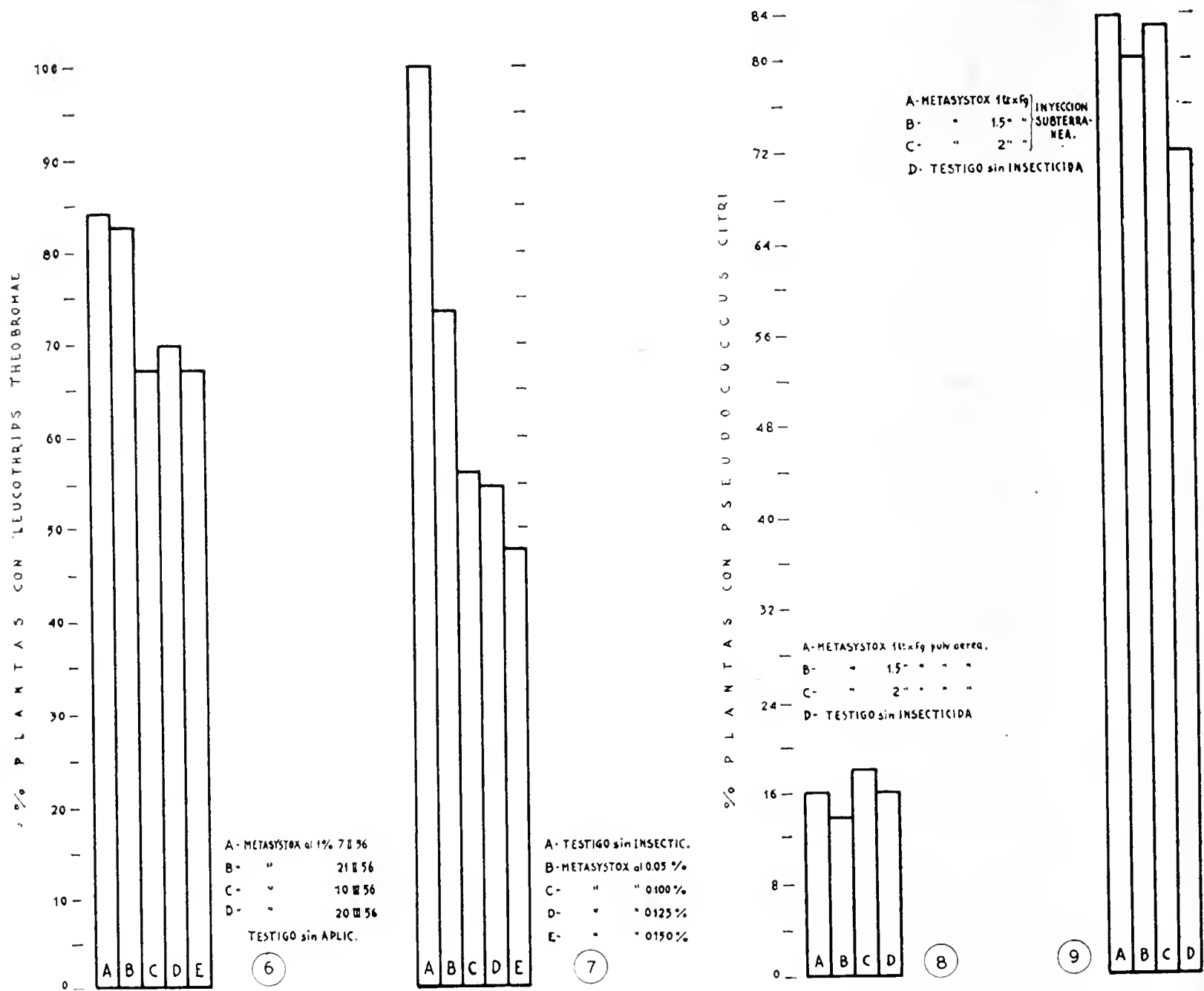


Fig. No. 6. Población de *Leucothrips theobromae* en el ensayo de época de aplicación de Metasystox (Lima, 1956).  
Fig. No. 7. Población de *Leucothrips theobromae* en el ensayo de diferentes dosis de Metasystox (Lima, 1956).  
Fig. No. 8. Población de *P. citri* en el ensayo de dosis de Metasystox conducido en la Hda. S. Juan Bautista. (Lima, 1956).  
Fig. No. 9. Población de *P. citri* en el ensayo de dosis inyectadas al suelo de Metasystox conducido en la Hda. S. Juan B. (Lima, 1956).

Nota: 1 fanega (fg) es igual a tres hectáreas aproximadamente.

CUADRO I. Número de Plantas con *Aphis* en el Ensayo en la Hacienda Huarabí Alto (Valle del Chillón), Lima, Perú.

Días desde la aplicación	Número de plantas con <i>Aphis</i>		
	Testigo	Systox	Metasystox
0	100	100	100
3	100	4	0
14	100	4	4
24	100	0	4
33	100	16	12
45	100	88	84

En el se vé que Systox y Metasystox mantienen una baja población de plantas con Aphis hasta los 33 días después de la aplicación.

En otro ensayo en el que se usaron los siguientes insecticidas:

A.- Systox	en pulverización aérea	165 cm <sup>3</sup> por Ha.
B.- Metasystox	" " "	330 " " "
C.- OMPA	" " "	330 " " "
D.- Primin	" " "	330 " " "
E.- Sytam	" " "	330 " " "
F.- Metasystox en inyección subterránea		330 " " "
G <sub>i</sub> - Thimet (3911) en polvo al cuello de la raíz		87 Kgs. " "
G <sub>ii</sub> -4739 (Bayer)	" " " " " " "	18 " " "
H.- Testigo sin insecticida.		

Los resultados se expresan en el Cuadro II.

CUADRO II. Porcentaje de Plantas con *Aphis gossypii* en el Ensayo Eomparativo de Insectícidias sistémicos en la Estación Experimental Agrícola de La Molina, Lima, Perú.

Días desde la aplicación	Número de plantas con <i>Aphis gossypii</i> sobre 100								
	Systox	Meta-systox	OMPA	Primin	Sytam	Metasys-tox in-yectado	Thimet (en polvo)	4739 (en polvo)	Tes-tigo
0	93	89	83	91	88	94	88	—	88
2	45	19	87	82	82	94	64	—	91
6	31	6	63	93	56	77	0	—	94
-10	10	0	65	99	22	70	8	—	99
16	8	0	71	100	47	71	0	—	100
25	15	4	77	100	58	75	0	—	98
30	26	9	79	100	60	87	16	—	96
37	28	2	81	97	64	92	0	Aplicado aqui	99
44	34	4	60	87	54	89	4	( 2) 100	89
55	54	12	90	99	80	95	20	(13) 92	86
63	50	18	93	99	72	100	4	(19) 92	93
67	92	61	88	95	93	100	20	(25) 64	98
74	100	93	100	100	100	100	80	(32) 100	99
81	89	73	100	100	100	100	48	(39) 88	100

NOTA.-Los números entre paréntesis en la columna del 4739, indican los días transcurridos desde la aplicación.

Como se vé Systox controló al *Aphis* con un poder residual de 25 días. Metasystox realizó mejor control y su poder residual fué de más de seis semanas (44 días). OMPA no ejerció un control satisfactorio aunque demostró cierta acción sobre el pulgón de la melaza entre los 2 y 10 días. Primin, excepto una ligerísima acción inicial que puede ser atribuída a "acción de contacto" y de ningun modo puede ser calificada de "sistémica", no ejerció control satisfactorio sobre el *Aphis gossypii*. Sytam controló bien al *Aphis* pero su poder residual no va más allá de 16 días. Metasystox inyectado no funcionó, posiblemente porque llegó a las raíces ya muy diluído en el agua del riego posterior a la aplicación. Thimet (3911 de American Cyanamid Company) fué aplicado en su formulación de 50% en carbón activado directamente al suelo abriendo un hueco en el costado del lomo del surco con la lampa y luego tapando, en la proporción de 87 Kgs. por Ha., ejerciendo un ligero control

los primeros dos días para luego convertirse, a los 6 días en un magnífico control con un poder residual de más de 9 semanas (67 días). Desafortunadamente la dosis aplicada es cinco veces mayor que la recomendada por los fabricantes y así estos resultados no son aplicables en la práctica pues resultaría muy caro. Un último producto probado contra *Aphis* fué el 4739 de Bayer que demostró, aplicado en la misma forma que 3911, pero a una dosis cinco veces menor, no controlar satisfactoriamente al *Aphis* aunque si se notó acción sistémica contra el pulgón de la melaza. Nuevos experimentos con estos dos productos están siendo llevados a cabo en la Estación Experimental Agrícola de La Molina, Lima, Perú, pero los resultados no están aun disponibles.

Por los buenos resultados obtenidos con Metasystox se ensayaron diversas dosis demostrándose, como se vé en el gráfico de la Figura N° 4, que a partir de la dosis recomendada por los fabricantes se acorta o alarga el poder residual a medida que se baja o se sube la dosis, respectivamente, pero también se demostró que una dosis muy baja no ejerce acción alguna sobre el pulgón de la melaza.

En cuanto a compatibilidad se ha encontrado que en mezcla con Arseniato de Calcio, Systox y Metasystox pierden algo de su poder, tanto inmediato como residual posiblemente debido a la reacción francamente alcalina de la mezcla. Sin embargo, en otro ensayo, se demostró que la reacción alcalina del rocío de la planta de algodón no ejerce acción marcada sobre Metasystox.

En cuanto a la destrucción del balance biológico ya es mundialmente conocido, y por éello no insistiré en esto, que Systox, Metasystox, Thimet y otros insecticidas sistémicos destruyen muy poco a los controladores biológicos del *Aphis gossypii* y que la disminución de ellos, que se nota en los campos tratados, es debida más a falta de alimentación que a acción insecticida. Ensayos realizados en el Perú demuestran que Metasystox destruye mucho menos controladores que otros insecticidas orgánicos tales como Aldrin, DDT, BHC, Endrin y Folidol.

Finalmente trataremos del ácaro *Paratetranychus peruvianus* y diremos que es bien controlado por Metasystox en dosis desde 365 cm<sup>3</sup> por hectárea como mínimo, aunque en el algodón Tangüis demora por lo menos cuatro días entre la aplicación aérea y la acción sistémica contra el ácaro.

La Figura N° 5 muestra que Metasystox al 0.1% controla bien al *P. peruvianus* pero necesita cierto número de días para destruirlo.

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## DISCUSSION

L. G. MERRILL, JR. Is metasystox used commercially in Peru?

J. E. SIMON F. Yes. More than 10,000 hectares were treated last season with metasystox in Peru.

S. S. EASTER. The speaker mentioned that after all treatments of chlorinated hydrocarbons there is an increase in aphids. I have noted that many fields of cotton treated with endrin have been free of aphids. Have you any observations on this line in Peru?

J. E. SIMON F. We have noted that fields treated regularly with endrin are free of aphids. However, we note that after three or four weeks aphids will increase. As the growing season is long in Peru we are trying to find an insecticide with a longer effective period.

# Bases para la Lucha en Gran Escala contra las Orugas en los campos de lino y Alfalfa en la Argentina

Por PABLO KOEHLER

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La lucha contra las orugas en superficies extensas constituye uno de los problemas mas graves de la agricultura Argentina.

En las estadísticas oficiales se calcula el daño producido por las orugas en una pérdida de dos a cinco por ciento como promedio. Esta cifra no sería excesiva, pero la situación real es distinta: muchos campos de lino de siembra temprana no sufren daño alguno, mientras las siembras tardías con frecuencia sufren una pérdida total. Para los propietarios de aquellos campos, que no pudieron ser arados más temprano, a menudo por falta de lluvias, la pérdida no alcanza el 2 a 5%, sino facilmente el 100%.

La lucha es dificultada por las grandes extensiones afectadas, las que siempre sobrepasan las cien hectáreas; en un caso específico un campo cerrado de una superficie de 2500 hectáreas se halló afectado. Tales extensiones, al ser atacadas, deben ser limpiadas en un lapso brevísimo, puesto que el agricultor observa la plaga recién cuando las orugas se hallan cerca del término de su desarrollo. Esto se explica facilmente por el rápido crecimiento de las orugas que cumplen el ciclo evolutivo del huevo a la larva en el término de 12 a 20 días.

Las variedades principales capaces de una reproducción en escala tal que pueden convertirse en plaga, pertenecen a las Noctuidae: *Heliothis obsoleta* F. (*Chloridea*, *Thyreion*), *Plusia* nu Gn. *Pseudaletia unipuncta* (Haw) (*Cirphis*), *Laphygma frugiperda* A&S; las *Agrotinae* de las variedades *Agrotis*, *Feltia* y *Peridroma* son de reducida importancia. Para los alfalfares debe agregarse como plaga específica la oruga de la mariposa *Colias lesbis* F, la que al igual que las especies nocturnas citadas, se desarrolla en forma completa en menos de 30 días.

El índice de infestación de orugas se determina mediante el "catcher", de manera de poder apreciar superficialmente, pero con rapidez el número total de isocas por hectárea, efectuando 20 redadas por cada 10 metros cuadrados aproximadamente. En presencia de una o varias especies, es posible recoger con veinte redadas entre 20 y 1500 orugas.

Datos exactos los suministra el recuento de larvas, proporcionando ocasionalmente cifras increíbles, como en el caso de Arruffo, Santa Fé, donde se estableció en un campo de lino la cantidad de 260 larvas por metro cuadrado de suelo, del tipo *Heliothis*. En Entre Rios se hallaron en un campo de avena 210 larvas de *Pseudaletia* en el suelo. En un campo de lino de San Carlos, Provincia de Santa Fé, se hallaron por metro cuadrado 310 de la variedad *Plusia* entre las cápsulas de semillas. Las orugas generalmente no se advierten cuando son menos de 20 por cada 20 redadas. (20.000 por hectárea). Al ser descubiertas en los últimos 3 a 4 días antes de su crisalidación, no alcanza el tiempo para procurar las máquinas, el material y el personal necesario para combatirlas.

El tratamiento de grandes extensiones siempre es difícil cuando se debe de evitar el daño de los sembrados por efecto de los vehículos, etc.; mucho más difícil aun, cuando se debe realizarse en forma apresurada, llegando a ser imposible al faltar las máquinas, insecticidas y la mano de obra necesaria. Es importante destacar además, que el agricultor jamas podrá controlar suficientemente tamañas extensiones y descubrir las orugas en su primera generación y sus primeros estados de desarrollo.

Desde el año 1933 hemos ensayado muchos métodos para lograr una rápida eliminación de las orugas en masa, como se ha logrado con las langostas, ofreciendo logicamente muchos atractivos la idea del *espolvoreo de tóxicos por contacto*. No es, sin embargo posible destruir las variedades de orugas nombradas por medio de espolvoreos pues se debería utilizar cantidades de polvo y concentraciones de veneno excesivas, lo que torna al tratamiento antieconómico y además peligroso.

Los ensayos demostraron, que la aplicación de tóxicos líquidos modifica ventajosamente esta situación, pero debido a la falta de agua con frecuencia resulta imposible tratar grandes superficies con este medio. Sin embargo estas posibilidades han sido fundamentalmente mejoradas con la utilización del "Mikron Sprayer" inglés.



La máquina aludida pulveriza normalmente unos 25 litros de producto por hectárea. Los 80 litros de contenido de su tanque permiten cubrir una superficie de dos hectáreas y media, las que son tratadas en forma de franjas de 25 metros de ancho por mil metros de longitud por corga. La velocidad de trabajo depende de la capacidad de la bomba. Generalmente un tanque de 80 litros es vaciado en unos 12 a 15 minutos.

El aparato funciona sin presión y distribuye las gotitas uniformemente en ángulo recto a la dirección de su movimiento. Sin ser afectadas por el viento, estas gotitas de tóxico quedan adheridas lateralmente y debajo de las semillas del lino, o sea exactamente en el lugar donde mayor será su eficacia debido a su efecto oral sobre las orugas.

En el curso de los últimos dos años se realizaron ensayos con "Mikron Sprayer" motorizados y se comparó su eficacia con otras máquinas, como también con Helicópteros sistema Bell y Sikorsky. Equipos de trabajo "Mikron Sprayer" trataron 1200 hectáreas de cultivos de lino y alfalfa, y los helicópteros además varios centenares de hectáreas de maíz y sorgo. Todas las parcelas de ensayo excedieron de una hectárea, contando además con réplicas y "tests", de manera que para cada ensayo se hallaban disponibles para control y observaciones por lo menos cinco hectáreas.

Se ensayaron los productos siguientes: DDT, HCH, Lindane, Chlordane, Heptachlor, Dieldrin, Aldrin y Toxaphene. Se eliminó el HCH para tratamiento de Alfalfa, por ser inconveniente para la alimentación de ganado lechero. El estado de experimentación con otros productos de reciente desarrollo aun no permite ensayos en gran escala.

En campos de grandes extensiones pudimos siempre lograr un control total, en cuanto sobrepasamos levemente de DL/95 y alcanzando pulverización perfecta. Esta destrucción total nos aparece fundamental para interrumpir el ciclo evolutivo, y para convencer al agricultor de la importancia de combatir a la plaga, por medio de las orugas muertas y las diferencias en las redadas entre parcelas tratadas y "no tratadas."

Pudiendo obtener el control de diversas plagas por métodos y dosificaciones idénticas es necesario limitar la proporción de los principios activos a lo más imprescindible, a fin de poder aplicar las mismas formulaciones a alfalfares. El propósito nuestro era evitar toda intoxicación de las crías de ganado, aves del corral, etc., además de reducir el costo de aplicación—manteniendo la eficacia total—por disminución de los principios tóxicos.

Para lograr estos fines, hemos ensayado mezclas sinérgicas con tal éxito, que pudo llegarse a una disminución del *material activo* hasta 330 gramos por hectárea, estimándose que esta reducción de veneno—con una mortandad del 100% en 24 horas—podrá muy difícilmente ser superada.

Para trabajos planificados futuros será recomendable fijar el tiempo para el tratamiento del lino en el período de la primera floración, mientras que los *alfalfares* deberán ser rociados después del primer corte, tan pronto los nuevos brotes midan algo más de 20 centímetros. El tratamiento de las flores trae como consecuencia una disminución en la formación de las semillas.

Grado de Eficacia de los Insecticidas Simples Contra las Orugas del Lino y Alfalfa.  
(Aplicación con Micron-Sprayer y 25/30 litros de agua por hectárea)

Producto	Principio activo Gms/Ha	Mortandad	
		%c	%
Aldrin	575	94	100
Lindane	600	99	100
Dieldrin	540	96	100
Toxafene	1575		100
Clordano	1500		100
Heptacloro	820		100

Efecto de Mezclas Sinérgicas  
(Aplicación idéntica)

Producto	Principios activos Gms/Ha	Cantidad total Gms/Ha	Mortandad
<u>Aldrin</u> DDT	<u>115</u> 250	365	94 - 100
<u>Dieldrin</u> DDT	<u>90</u> 250	340	97 - 100
<u>Clordano</u> DDT	<u>120</u> 500	620	100
<u>Heptacloro</u> DDT	<u>82</u> 250	332	100
<u>Heptacloro</u> Toxaphen	<u>82</u> 360	712	99 - 100



# Wheat Blossom Midges on Broadbalk, Rothamsted Experimental Station, 1927-56

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## ABSTRACT

The author, after explaining one purpose of this study, describes the methods used to establish the annual populations of the larvae in the wheat and to ascertain, by rearing the larvae, the emergence dates of the midges and parasites, together with their relative abundance.

Assessments of larval populations should be based on the floret rather than the ear-infestations. Tables indicate the numbers of larvae that result in the different levels of shrunken grain by *S. mosellana* and loss of grain by *C. tritici*.

The mean periodicity of *S. mosellana* is 3.14 years, while that of *C. tritici* is 2.9 years. These suggest some connection with the 3.2 factor derived from other animal population studies and which appears to be a cycle in ultra-violet radiation.

Similar rhythms appear in the relative parasitism.

When both wheat blossom midges are considered together there appears to be a tendency for larger numbers than usual to occur at approximately 5-year intervals. This may be due to climate. Local weather however frequently prevents this happening in any particular locality.

Temporary cessation of activity takes place among the fully fed larvae in the ears during dry seasons and a much longer diapause, extending up to 13 years in *S. mosellana*, occurs among the larvae in the soil.

## INTRODUCTION

Originally this study was one of a series of long-term studies designed primarily to collect information regarding the fluctuations of insect populations as they occur in nature and secondarily to provide hints of the factors involved so that these could be tested experimentally. The immediate objective was to develop methods whereby yearly assessments of the numbers of certain pests could be made comparatively accurately and be put on a routine basis. If this could be achieved the results would indicate, 1. to what extent yearly control by insecticides was necessary and desirable, 2. whether or not the forecasting of outbreaks was possible and 3. would perhaps lead to the assessment of the damage caused.

Unfortunately this project never attracted sufficient interest to enable it to be developed on a co-operative basis, so one by one the studies faded out leaving only the one dealing with the Wheat Blossom Midges, *Contarinia tritici* Kirby and *Sitodiplosis mosellana* Géhin. This particular study has now been going on for thirty years so it is perhaps excusable to bring forward again a matter that was first brought to the attention of the International Congress at Madrid in 1935.

The classical field<sup>1</sup> Broadbalk on Rothamsted Experimental Farm at Harpenden, Herts., which has carried autumn-sown wheat each year since 1843, was chosen for this study since it was thought that an equilibrium would have been set up between the midges and their parasites and predators. As a consequence any fluctuations in populations would probably be due in the main to the local weather or to changes in climate, acting either directly on the midges, or indirectly through their parasites or through their food plant.

In order to ascertain the annual level of larval infestation, ten plots of wheat have been sampled each year, fifty ears being taken from each plot so as to make a composite sample of 500 ears for the field. In addition this method has allowed a comparison to be made of the larval infestations of wheat under different manurial treatments. Since the 5-year cycle of fallowing was initiated, the plot samples have been subdivided, so that a comparison of the effect of fallowing for one year could be ascertained. For the first 14 years of the study the sampling date was chosen by field and insectary observations of the midges' emergence and by field observations of the subsequent growth of the larvae.

<sup>1</sup> This field is divided into 17 parallel half-acre plots each receiving a distinct manurial treatment year after year. In 1925, the field was divided across the plots into five equal sections so that weeds could be controlled by fallowing part of the field each year. By 1934-5, a five year cycle had developed; each year a different section is fallowed and the other four-fifths are under wheat. In 1954, the top half of Section I was reverted to continuous wheat. Since 1899 the wheat variety has been Squareheads Master or Red Standard.

However, it was uncertain that the maximum population was still up in the ears on the sampling date, so a system of presampling was started in 1941 and has been continued to date. This involves taking frequent samples of 50 ears on one plot (for a sequence of five years, on another for the next five years, and so on) from the time the larvae are small until they have left the ears. From the numbers of larvae and the stage of growth reached the correct date for the main sample is obtained.

All the larvae that are extracted from the year's main sample are immediately placed in emergence pots, the larvae from each pair of plots being put together but the two species kept separate. Thus each year's sampling results in five pots containing *C. tritici* and another five with *S. mosellana*. On completion of the counting all the pots are removed to the insectary where they are retained for some years. In this way the subsequent emergence dates of the midges and their parasites, as well as their relative abundance, are ascertained in subsequent years. For many years the dates of emergence in the insectary closely approximated that in the field, but in 1956 emergence of *C. tritici* in the field started days before that in the insectary.

Many results of this study, as well as tables showing the annual infestation on the ten plots of Broadbalk from 1927-40, have already been published. Full references to these and the main points resulting from this study are given by Barnes (1956).

In this contribution attention will be focussed on the following points: 1. the necessity for presampling in order to ascertain the correct date for sampling, 2. the relationship between the numbers of larvae of *S. mosellana* or *C. tritici* and the number of grains shrunk or lost to each species respectively, 3. the periodicity of the two species and their parasites, and 4. the two periods of cessation of activity exhibited by the larvae.

### THE NECESSITY FOR PRESAMPLING

The amount of work involved in extracting and enumerating the larvae from an adequate sample of ears makes it desirable to sample each year on one day only. A preliminary approximation of this date can be made by allowing roughly 20 days to lapse after the peak of the midges' emergence which usually coincides with 50 per cent ear-burst. However since emergence, and consequently oviposition and larval growth, is prolonged and since rain enables the individual larvae to leave the ears as they complete the feeding stage of their development, it is quite possible to obtain larval infestation figures that do not comparably represent the populations of either species over a sequence of years. Therefore, a presampling method has been devised; small samples are taken at frequent intervals to determine larval numbers and size throughout the period they are up in the ears. This enables the exact date for the year's main sample to be determined and also, if the larval descent happens to occur before the main sampling can be done, a correction can be applied. If the accuracy level of the infestation figure required is not too high, the main sampling might even be omitted.

Two examples of different types of larval descent will be given: the sudden one that occurs in dry seasons, and the gradual one that takes place in wet seasons.

Fig. 1 shows the quantity of larvae of *C. tritici* extracted from fifty ears gathered at random on July 8, 11, 14, 16, 20, 23, 27, 30, August 2, 5, 8, 10, 12 and 16 on Plot 11 in 1955. Although 1186 (from 25 ears only) and 2676 larvae are in the first two vials it is obvious that, especially on July 8 (1st vial), the larvae were too small to be certain all were found. By July 14 and 16 (vials 3 and 4 containing 3358 and 3585 larvae) the larvae were thought to be large enough for accurate extraction from the ears. Two further days were allowed to lapse before taking the main sample for the year, so that sufficient growth would have taken place to enable the majority of the larvae to develop to the adult stage in the emergence pots. The main sample was then taken on July 18 and the larvae dealt with as mentioned above. From July 14—August 12 the number of larvae per 50-ear sample varied from 4277 to 2483 with an average of 3238, including those 3040 taken from the same plot as part of the main sample of July 18. This range is assumed to be the sampling error and that no appreciable larval descent had taken place up to August 12 throughout the dry spell.<sup>2</sup> This is supported by the fact that the average number of larvae per infested floret

<sup>2</sup> In 1955, there was a prolonged period without rain during the time when the larvae were in the ears. Only traces of rain were recorded between July 8 and August 7; on August 10 and 11, 0.03 and 0.08 inches were recorded respectively. An appreciable amount, 0.46 inches, fell on August 13. The effect of this exceptionally dry period was that the larvae were unable to leave the ears and remained upon the wheat much longer than usual.



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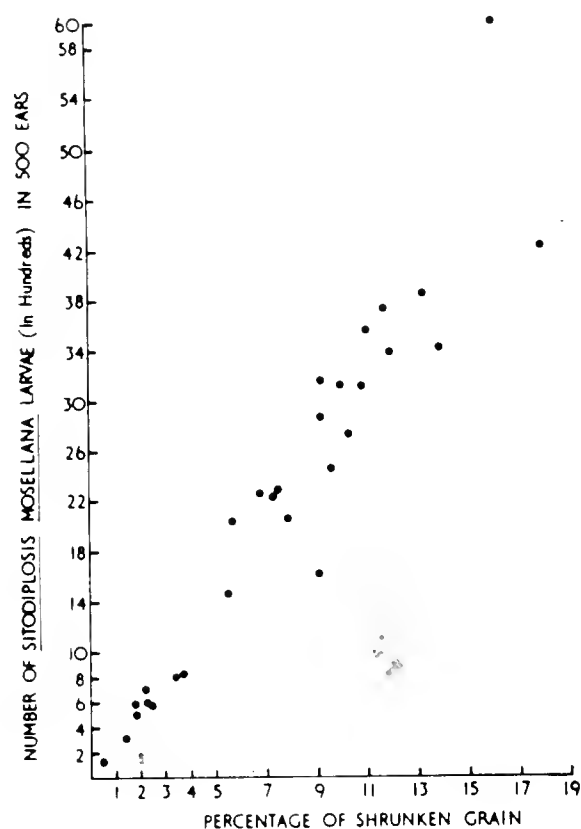
Fig. 2 shows the numbers of larvae of *S. mosellana* in the same samples. Vials 1 and 2 contain 66 (from 25 ears only) and 106 larvae that were present on July 8 and 11. The larvae were still too small for accurate extraction. The numbers present from July 14 to August 12 (vials 3—13) varied from 198 to 343 with an average of 264, including those 207 taken from the same plot as part of the main sample for the year on July 18. Again it is reasonable to suppose that the range can be taken to represent the sampling error and that no appreciable descent had taken place up to August 12. The average number of larvae per infested grain is of no value since this species is almost solitary. The last vial containing only 13 larvae indicates the sudden descent after the rain on August 13.

In 1950, a wet season, the picture obtained by presampling was utterly different. The data are shown in Table I. On July 1 the larvae were too small for accuracy, yet on July 9 when the larvae were of reasonable size the descent had already started. This early descent took place because of the wetness of the period from July 1 to 24 when the larvae were able to leave the ears as they completed feeding. Rain fell on 16 days and ranged from 0.12 to 1.40 inches on nine of them with lesser amounts on seven days. By July 24 practically all of the larvae had left the ears. The curves of descent of the two species represent the dates at which the larvae reached full growth since they were able to leave the wheat

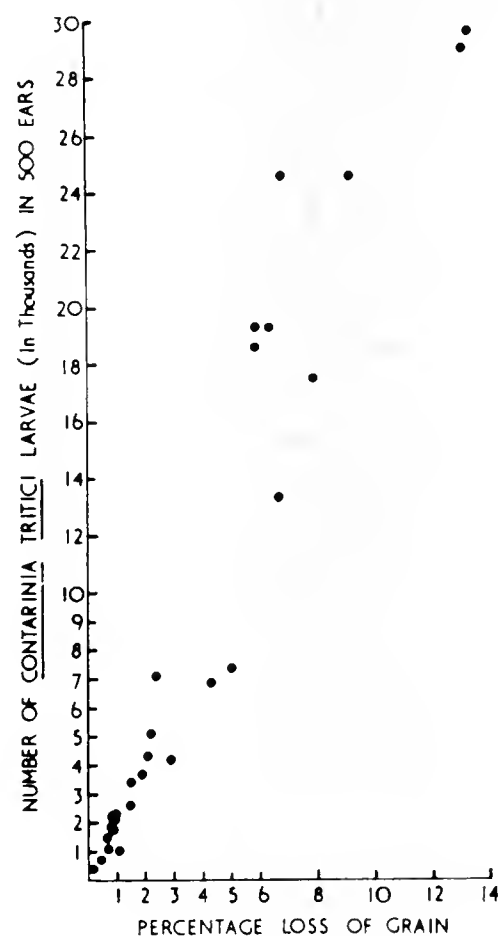
Date	Number of <i>C. tritici</i>	Average number per infested floret	Number of <i>S. mosellana</i>
July 1	1468	9.2	238
July 9	1344	6.8	393
July 10	791	5.0	379
July 12	491	3.9	324
July 14	228	2.4	266
July 17	73	2.0	231
July 19	113	2.2	179
July 24	30	1.8	51

without any delay. They may also be assumed to reflect the oviposition curves that had previously taken place.

The main sample was taken on July 10 so an adjustment had obviously to be made for the figures obtained for *C. tritici*. Those obtained for *S. mosellana* were so close to the beginning of the descent that correction was unnecessary. Figs. 5 and 6 contrast the

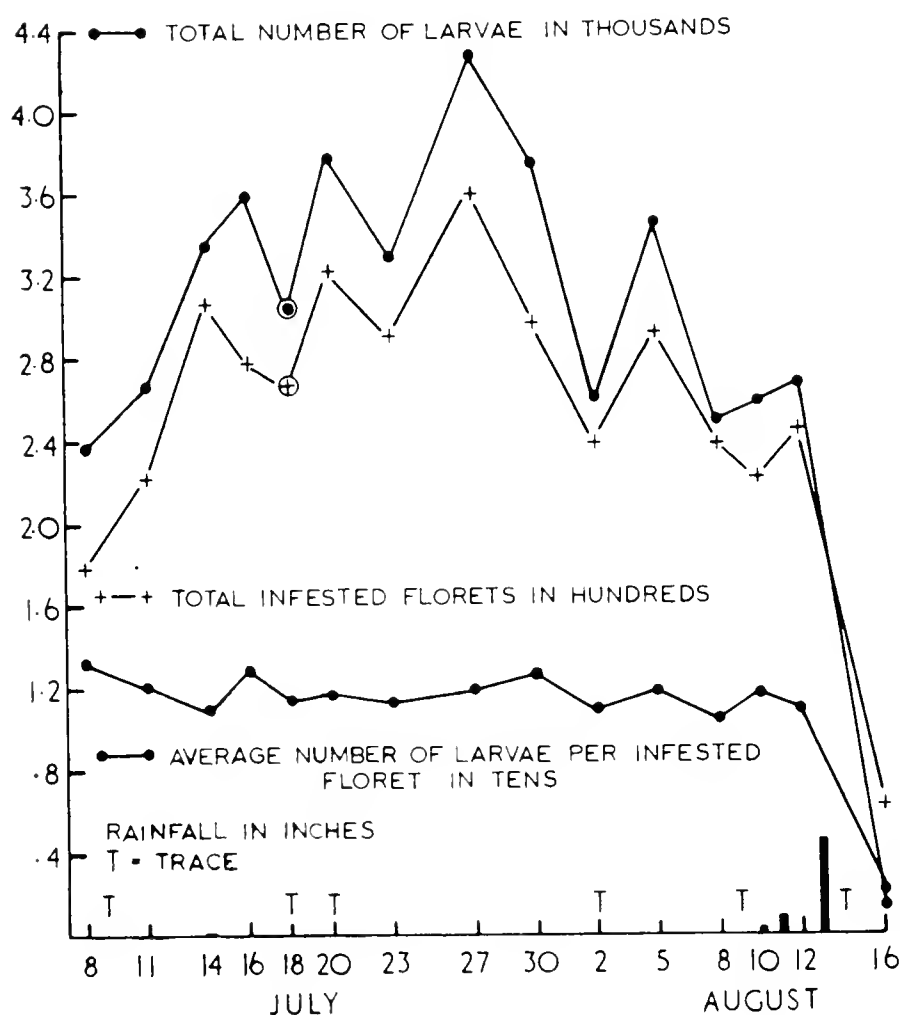


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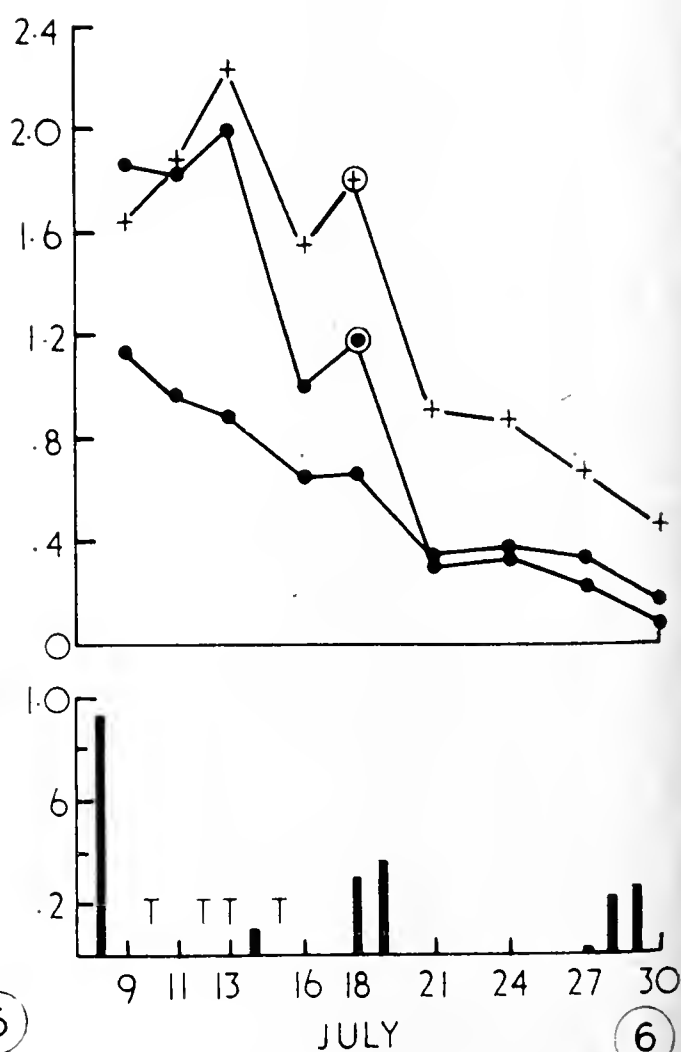
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*Contarinia tritici* (Kirby)  
1955



5

*Contarinia tritici* (Kirby)  
1956



6

Figs. 3, 4. Relationship between the number of larvae and the percentage of shrunken grain. Fig. 3, *S. mosellana*. Fig. 4, *C. tritici*.

Figs. 5, 6. Rainfall during presampling period in relation to numbers of *C. tritici* larvae extracted from wheat, on the basis of 50-ear samples, 1955 and 1956.

numbers of *C. tritici* larvae extracted during presampling in 1955, a dry season, and in 1956, a wet one similar to 1950. Presampling figures obtained in other years have shown that although the descent of the larvae may start in suitable weather it may be halted for a few days by the onset of dry weather, only continuing when the necessary conditions again arise.

#### THE RELATIONSHIP BETWEEN THE NUMBERS OF LARVAE AND THE NUMBERS OF GRAINS SHRUNKEN AND LOST

Tables have already been given (Barnes, 1956) showing the percentage ear infestation in relation to the percentage floret or grain attack. They indicate clearly that, in order to get an accurate idea of the yearly damage, it is essential to assess the percentage floret or grain attack. The less onerous method of recording merely the percentage ear attack gives only a very rough measure of the extent of the damage. In the case of *C. tritici* it seems that only when the percentage ear infestation exceeds 70 is there likely to be a 5 per cent or more grain attack. Higher ear infestations by *S. mosellana* normally occur more frequently than by *C. tritici*. However, in 19 samples that showed a 100 per cent ear infestation the percentage grain attack ranged from 8.5 to 17.

The Broadbalk study has also enabled tables to be drawn up indicating the numbers of larvae necessary to cause different levels of damage due to grain being shrunk by the presence of larvae of *S. mosellana* or lost by the presence of larvae of *C. tritici*. These relationships are shown in Figs. 3 and 4. Because the larvae of the former species are almost solitary, while those of the latter are gregarious, fewer larvae of *S. mosellana* can cause significantly high levels of damage. These tables and figures may be of use in determining the extent of infestations directly from the numbers of larvae found. This would be very labour-saving if an accurate method (e.g. by the suspension of the ear sample in the presence of some vapour) was devised to cause the larvae to leave the ears of their own accord.

#### THE PERIODICITY OF THE TWO SPECIES AND THEIR PARASITES

It was suggested (Barnes, 1932) that, considering both species together, rhythmic fluctuations in numbers might be expected to occur with peaks about every fourth, fifth or sixth year.

Twenty years after the commencement of the study (Barnes, 1947), there had been two large peaks, one in 1931 and the other in 1946, as well as two minor ones in 1935 and 1941. The regularity of these peaks had been broken by the absence of a peak in 1936. It has been postulated that the abnormal precipitation and subsequent hot week, experienced in that year at the time of the midges' emergence and oviposition, might have accounted for the unexpectedly low numbers of larvae in the wheat. But in addition to this, the wheat data on Broadbalk, regarding the effects of manurial treatments and so on, were strange as a whole although other fields on the same farm gave normal results.

Barnes (1953) pointed out that the suggested five-year rhythm demanded that 1931, 1936, 1941 and 1951 should have been peak years, but that in 1936 and 1951 the actual numbers fell short of expectation. In 1951 the usual close parallelism of the midges' emergence and ear-burst did not materialise and this could have accounted for the diminution in numbers on this field. It is interesting to interpolate here the fact that there was a serious outbreak of *C. tritici* in southern Eire in 1951.

These two shortcomings in 1936 and 1951 emphasise the weakness of confining such a study as this to observations on one field if one considers that the rhythm is basically correlated with a climatic rhythm (see below); local weather might prevent the expected from happening.

In the same paper (*loc. cit.*) it was stated that the data so far obtained indicated peaks of abundance every fifth year and that the next might be expected to occur in 1955 or 1956. The actual data obtained shows that from 1954 to 1955 the numbers of *C. tritici* rose from 4,179 to 24,604 and those of *S. mosellana* from 500 to 2,232. In 1956 the numbers of *C. tritici* fell slightly to 18,656 (adjusted), whereas those of *S. mosellana* fell precipitously to 105. In the insectary, relative parasitism of the latter species was 97%, whereas that of *C. tritici* was only 22%.

If one considers the two species separately, a different picture is obtained. Mr. H. F. Church of Berwick-on-Tweed who has examined my data has informed me (*in litt.*) that

"the mean periodicity for *C. tritici* is 2.9 years and for *S. mosellana* 3.14 years. These figures depend of course on the accuracy of the sampling methods and whilst not in perfect agreement with the 3.2 factor are nevertheless suggestive". The 3.2 factor is derived from fish (and several mammal and bird) population studies and "appears to be a cycle in ultra-violet radiation that has been traced through a wide range of meteorological phenomena, the annual variations of magnetic activity to a 39 months' variation in total solar radiation". Mr. Church goes on "Assuming the true factor to be between 3.20 and 3.25 years recurrences will hit the same month of the year between 13 and 16 year intervals. I think this is the explanation of the emergences of the so-called 17-year Cicada which in eastern United States emerges in vast numbers at intervals of 13 to 17 years dependant upon latitude. *C. tritici* shows somewhat similar behaviour. Thus great abundance from 1929-31 is repeated 1945-47. In the case of *S. mosellana* this rhythm is probably distorted by the long period of years over which the insects emerge".

Observations in the insectary, on material collected from the field, have shown that rhythmic fluctuations in larval populations of the wheat blossom midges are followed by similar rhythmic fluctuations of their parasites.

Finally, I believe the suggested rhythm is associated with some widespread climatic one that results in 'good' and 'bad' years for the reactivation of the cocooned larvae in the soil during the early spring and as a result the subsequent emergence of more or fewer midges.

#### THE TWO PERIODS OF CESSATION OF ACTIVITY EXHIBITED BY THE LARVAE

The first period of cessation of activity exhibited by the larvae occurs up in the ears during dry weather when the larvae have finished feeding but are unable to descend to the soil. When this happens to the gregarious larvae of *C. tritici*, what might be termed a 'congelation' of larvae results. One finds the larvae and apparently the sap in which they have been bathed congealed into a dry mass. If this is placed in water or weak alcohol, the sap dissolves immediately and the larvae start moving about actively. On the other hand, when the solitary larvae of *S. mosellana* are overtaken by such weather conditions and cannot move from the ears to the soil they assume a transparent skin. Apparently this is either specially secreted or is the outer larval skin, but it does not bear the sternal spatula which is retained by the larva within this skin. There is air between the skin and the larva as is easily demonstrated by placing it in water where it floats. However, in water the larva quickly becomes activated, comes out of the skin and sinks leaving the empty skin floating so long as the air bubble remains in it.

The second period of cessation occurs when the larvae are in the soil. After the larvae have completed their feeding stage on the wheat they descend to the soil in which they spin cocoons. They remain as dormant larvae in these until their reactivation shortly before pupation, after which emergence soon takes place. Since both species can be considered univoltine, although *C. tritici* has a partial second generation in most years, one would expect the whole of each year's larval population to develop, apart from mortality, into flying midges the following year. This in fact does not occur. In the case of *C. tritici*, although the overwhelming proportion does emerge the following year, some do not complete development until the second year after they have left the wheat, while a few do not appear as adult midges until the third and even the fourth year.

In the case of *S. mosellana*, adults continue emerging from one year's larval population for as long as thirteen years. The most interesting point about this prolonged larval existence in cocoons in the soil is the fact that the percentage emergence is not always proportionately lower as the years go by. Table II shows the numbers of *S. mosellana* larvae collected annually from the main sample during 1939 to 1955 and the numbers of midges and parasites that emerged from them over the period 1940 to 1956. It will be seen that the emergences do not steadily decline. Frequently more midges and parasites emerge in the second year than in the first, while occasionally most emergences take place in the third year and once even in the fourth year after the larvae have entered their cocoons. It is interesting to note that the emergence curves of any one year coincide irrespective of the duration of the diapause.

The reason for this prolonged cessation of activity is not at all clear. It has been suggested by K. K. Nayar that the onset, as well as the different periods for which any particular



TABLE II. Numbers of *Sitodiplosis mosellana* and Its Parasites Emerging in the Years Following That in which the Larvae were Collected from Wheat Ears.

Number of larvae	Year of collection	Year of emergence*																
		1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956
1676	(1939)	192	44	44	100	3	2	2	2	6	0	0	1	0	0	0	0	0
2342	(1940)	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		-	543	236	79	1	8	12	1	15	0	0	1	0	0	0	0	0
2836	(1941)	-	412	91	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		-	-	516	139	148	120	93	27	24	5	5	2	2	2	1	0	0
2131	(1942)	-	-	468	76	26	13	3	2	0	0	0	0	0	0	0	0	0
		-	-	-	281	22	182	84	13	10	3	4	4	7	5	0	3	0
2462	(1943)	-	-	-	337	32	12	28	0	0	0	0	0	0	0	0	0	0
		-	-	-	-	50	108	179	35	2	2	2	4	1	8	2	0	0
137	(1944)	-	-	-	-	2	0	0	5	0	0	0	0	0	0	0	0	0
		-	-	-	-	-	15	14	0	5	2	2	2	0	0	0	0	0
3693	(1945)	-	-	-	-	-	0	9	0	0	0	0	0	0	0	0	0	0
		-	-	-	-	-	-	155	28	43	4	81	6	0	6	6	6	0
4041	(1946)	-	-	-	-	-	-	25	41	8	1	48	2	1	0	0	0	0
623	(1947)	-	-	-	-	-	-	-	53	335	21	71	26	9	50	2	3	0
		-	-	-	-	-	-	-	53	29	1	7	0	1	2	2	3	0
2330	(1948)	-	-	-	-	-	-	-	-	5	8	7	0	7	28	0	0	0
		-	-	-	-	-	-	-	-	45	11	0	1	0	0	5	0	0
819	(1949)	-	-	-	-	-	-	-	-	-	61	87	1	8	82	5	9	0
		-	-	-	-	-	-	-	-	-	95	137	5	5	13	2	0	0
3276	(1950)	-	-	-	-	-	-	-	-	-	-	14	3	21	111	6	58	4
		-	-	-	-	-	-	-	-	-	-	45	5	6	12	0	4	0
3500	(1951)	-	-	-	-	-	-	-	-	-	-	-	7	7	210	10	134	16
		-	-	-	-	-	-	-	-	-	-	-	207	57	304	10	65	35
1519	(1952)	-	-	-	-	-	-	-	-	-	-	-	-	2	125	48	195	83
		-	-	-	-	-	-	-	-	-	-	-	-	201	209	94	119	103
3279	(1953)	-	-	-	-	-	-	-	-	-	-	-	-	-	64	15	248	72
		-	-	-	-	-	-	-	-	-	-	-	-	-	166	26	63	42
506	(1954)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	59	78	60
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	168	92	276
2294	(1955)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	17
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	47
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	188

\*Upper figure in each row refers to adults of *S. mosellana*, lower figure to parasites.



larva remains in diapause, may originate from some endocrine activity. The adult *S. moselana* has exceptionally large corpora allata. This activity in turn may be connected in some way with the moisture conditions occurring when and where the particular larva is actually feeding and developing, i.e. the micro-climate surrounding the larvae in the wheat ears. The termination of this prolonged larval existence may be due to some climatic change in the early spring when the larvae become reactivated, leave their cocoons and can be seen moving about in the soil prior to pupation.

Whatever causes the onset or termination, some years, for example 1945 and 1946, 1950, 1953 and 1955, are 'good' years for emergence, while other years are 'bad' ones. In the 'good' years as one would expect larger numbers of larvae are usually obtained in the sample (Table II).

The year's flight is a composite one, consisting of individuals derived from larvae of up to the previous thirteen years (or generations). The consequent back-crossing among the midges might be expected to strengthen the population.

A further result of the diapause is that it reduces considerably the possibility of the species being exterminated by any catastrophic happening. On the other hand, massive populations can build up in the soil and consequently outbreak years do occur.

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# Ecological Studies on Vertical Movements in the Life Cycle of *Phyllophaga*<sup>1</sup>

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## ABSTRACT

The depths of occurrence of *Phyllophaga* larvae and adults were recorded in weekly diggings during the ten years, 1933-42, covering three complete three-year life cycles for each of the three broods. Soil temperature and moisture were recorded and their influence on vertical movements of the insect traced. The results are given of an experiment on the survival of newly hatched larvae in soil tanks held under a series of constant conditions of soil temperature and moisture.

The life cycle and activities of *Phyllophaga* was found to be very definitely governed by soil temperature and more especially soil moisture. The preferred levels at which white grubs feed and pupate is usually selected at the midpoint between hygroscopic coefficient and moisture equivalent of the soil, although they may feed, if necessary in dry weather, nearer the point of hygroscopic coefficient of the soil. The grubs exhibit a remarkable sensitivity to the soil moisture content during their summer feeding activities and may move upward or downward to select the preferred level of soil humidity after each periodic precipitation.

The hibernation of grubs in all stages of development as well as the overwintering of the adults is always nearer the level of the moisture equivalent of the soil.

The eggs and the newly hatched larvae of *Phyllophaga* are extremely sensitive to desiccation and if these stages in the life cycle correspond to the soil moisture deficiencies below the minimal levels of preferred soil moisture, a great reduction in the numerical population of a given brood may take place.

In severe drought years the population of white grubs was so much affected that the reversal of economic importance of broods took place, favoring those that escaped the drought effect in the younger stages of grub development.

Soil insects, because of their subterranean habits—thus being hidden from facile observation—constitute one of the most difficult problems and continue to present a formidable hindrance in basic entomological research. For that reason alone, their general life histories and biologies are indeed inadequately known. Much less is known of their behavior on the background of ramification and interaction of various ecological factors, which play an important, if not vital, role in the life economy and bionomics of an insect that habitually selects the soil medium, where it spends at least part of its life history in the course of individual development.

The subterranean forms or stages of most of the soil insects are extremely sensitive to slight variations in temperature and soil moisture of their micro-habitat, and once taken from their preferential micro-climate, they soon perish due to rapid desiccation or to some other causes of exposure. This phenomenon often prevents laboratory studies of soil insects away from their natural habitat, and the best efforts to duplicate natural conditions under modern laboratory facilities have not always proved successful.

Insect behavior in soil is an expression of an inherent reaction of a given species to the influence of the environmental complex presented by the soil medium. Soil in itself is an extremely complex mixture, consisting of different sizes of weathered particles of rocks mixed with organic matter in various stages of decomposition, acted and interacted upon by diverse micro-organisms in the presence of air and different amounts and types of water found in the ground of local areas. Soil is a constantly changing product of the actions and interactions of climate, vegetation and micro-organisms on the constituent parent material, characterized and conditioned by the past geological history and relief of terrain.

Soil, therefore, is not to be considered as an inert matter. On the contrary, it is a highly complex living thing. A handful of soil is teeming with life. Consequently, the interactions of the physical as well as chemical and biological processes of living soil, on

<sup>1</sup>Paper No. 3749, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota.

the background of its texture and structure, determine the suitability of the environment for the living processes of any species of soil insect.

A complexity of interactions of many physical, chemical and biotic factors, such as temperature, moisture, evaporation, light, color, gases, parasites and others, too well known to be listed here but which are important in the vital processes of soil, further determine the fitness of a given soil as a selected habitat for insect life as well as for the distribution and relative abundance of soil insects.

Since the building processes of the soil through the centuries have not been uniform over any large area, there are great local variations of terrain and soil. This, together with the great variation in local ground cover by vegetational communities which provide food and abode, still further modifies the habitat of insects and actually determines the presence or absence as well as abundance of a given species of insect in the area.

All this, in turn, only indicates the complexity of entomological problems associated with the studies of ecology and biology of soil insects. Many difficulties and variabilities, as well as perplexities and disappointments, are expected to be met with any phase of entomological research of soil insects in addition to sheer arduous physical labor required in the process of such studies. These are possible reasons why basic studies on soil insects have been neglected for so long. Nevertheless, in the last quarter of a century marked progress has been made.

In recent years, several laboratories have devoted some attention to the studies of biology and ecology of Phyllophaga, at least temporarily, but only a few of them have shown sustained effort for any length of time. This may be due to the physical difficulties involved in such studies. The Minnesota Agricultural Experiment Station has been interested in the problem of white grubs for some time, especially since 1932. Several ecological phases of the problem received considerable attention with special emphasis on the periodic fluctuation of Phyllophaga population. The more important of these studies included the qualitative and quantitative as well as seasonal distribution of Phyllophaga over the state by various broods, preferential ground covers for oviposition by June beetles, vertical and lateral migrations of white grubs and adults, and several other phases.

The ecological studies of vertical movements in the life cycle of dominant species of Phyllophaga started in the spring of 1933 and were carried on until the fall of 1942, thus covering three complete three-year life cycles for all three broods, "A", "B" and "C", the designations now well established in literature since the early studies of white grubs by J. J. Davis (1913-22). The species dominant in the area of St. Paul, Minnesota, are *Phyllophaga fusca* and *Ph. rugosa*, on which most of the present data are based. Species which are fairly common and may be locally damaging are *Ph. tristis*, *Ph. drakei*, *Ph. implicita*, *Ph. anxia*, *Ph. illicis* and *Ph. nitida*. Other species encountered but less common include *Ph. crenulata*, *Ph. futilis*, *Ph. inversa*, *Ph. marginalis*, *Ph. forsteri* and a few others.

The vertical movements of white grubs and adult beetles were traced by weekly diggings for ten years, using as a standard unit a frame enclosing one fourth of a square meter, or 2.67 square feet, of surface. Most of the diggings were made to the depth of 36 inches in a heavy silty-loam soil and down to 74 inches in sandy soil, during the early spring, fall and winter. During the summer months, when grubs feed in the upper layers of soil, the diggings were made only to the depth of 20 inches. During the first two years, weekly excavations were made throughout the entire year, including the winter months, with the atmospheric temperature often below zero, and on some occasions  $-16^{\circ}$  F., chiseling the frozen soil bit by bit, recovering various stages of Phyllophaga and recording their exact location.

During the other eight years digging usually started early in the spring, before the upward movements began and into late fall, when most of the larvae had moved downward for hibernation. Occasional excavations were made in the winter months to ascertain the depth at which the larvae in various stages of development and the adults could be found after the ground was thoroughly frozen. These upward and downward movements of grubs were correlated with soil moisture and seasonal soil temperatures.

Soil samples were taken for soil moisture determinations, usually at the levels of 1 to 2, 5 to 6, 9 to 10, 17 to 18, and 23 to 24 inches. Occasionally, depending on the season and other conditions, the soil moisture determinations were made at other levels. The

percentages of soil moisture content were determined by heating the collected sample of soil for 72 hours at 105°C. in an air dry oven.

The records of soil temperature were made available by daily thermocouple readings which were taken throughout the year from the surface to the depth of six feet at intervals of 2 inches in the upper 6 inches and then downward at 4 inch intervals. In addition, soil temperature records were taken at the same levels as the samples for soil moisture determinations. Records of the weekly totals of precipitation were obtained from a nearby meteorological station.

At the beginning of these studies, all of the soil, as it was dug out by thin layers from the sample holes, was carefully sifted for the recovery of various stages of *Phyllophaga*. It was soon found impractical. Later the soil was carefully examined by hand as it was removed in thin layers and spread out by a trowel. By this method it is possible to detect the smallest stages of white grubs and eggs and no difficulty at all was encountered in recovering the larger forms of *Phyllophaga*.

In examining the records it was found that in silty-loam soil, having a clay and light gravel subsoil, the grubs move downward for winter hibernation to about 30 inches below the surface of the ground, the majority overwintering between 15 and 26 inches below the surface; while in sandy soil they may go as far down as 62 inches, the average depth being between 30 and 36 inches.

The beginning and rate of downward migration of white grubs in the fall varies considerably with the season and definitely depends on the amount of early fall precipitation, the soil moisture and the attendant lowering of the soil temperature. In 1933 (Fig. 1), the month of August was extremely dry with soil moisture only 2.42 per cent in the upper two inches and 3.41 percent at the level of 9-10 inches. With the weekly precipitation of 1.5 inches between September 7 and 14 the soil moisture increased to 16.61 percent in the upper two inches and at the level of 18-19 inches to 11.57 percent. This immediately stimulated the grubs in penetrating downward. With the additional precipitation in the following week, practically all grubs in the second year of development moved downward below the level of 15 inches from the surface for winter hibernation. The turnover of soil temperature at the depths of 72 inches and upper levels occurred just about the same time. However, the soil thermocline reversal of the autumn does not always coincide with the downward movement of the grubs.

Research data indicate that fall migration of white grubs is more dependent on the moisture content of the soil, which in turn depends on periodic precipitation. Thus in 1934 (Fig. 2) the turnover of soil temperatures took place between September 3 and 10, while the downward movement of the grubs in the first year of their development started more than a month later, between October 5 and 18, with the soil moisture content of 16.43 percent at the depth of 9 to 10 inches. Records of other years give the same pattern.

There is apparently no great difference in the initial downward movements between the grubs of the first and the second year of development, although the older, and especially the more physiologically mature, grubs may start downward movement somewhat earlier than the less developed grubs. In reaching positions suitable for overwintering, the grubs make earthen cells in which they spend the winter months until their upward movement early in the spring.

Actual measurements of the relative amount of available moisture in the hibernating earthen cells vary between 14.65 percent and 19.24 percent, in a soil having a moisture equivalent of 18.6. It appears, therefore, that grubs overwinter in a nearly saturated atmosphere and in contact with available soil moisture. The grubs at the normal levels of hibernation are never found frozen, although they are sluggish and flabby and are always completely defecated. The grubs may become frozen if they are caught in the upper layers with winter soil temperature of about  $-4^{\circ}$  or  $-5^{\circ}$  C., which is usually lethal to white grubs. However, the adult June beetles, which spend the winter at a much shallower depth, are often covered with ice crystals, within their earthen cells, but in only a few minutes when they are warmed up they slowly begin to move their appendages.

In the spring, the upward movement of grubs begins in the latter part of April, in the vicinity of St. Paul, and grubs appear in the first few inches of soil about the first week of May. Of course there is a slight variation from year to year. It was noted that the older



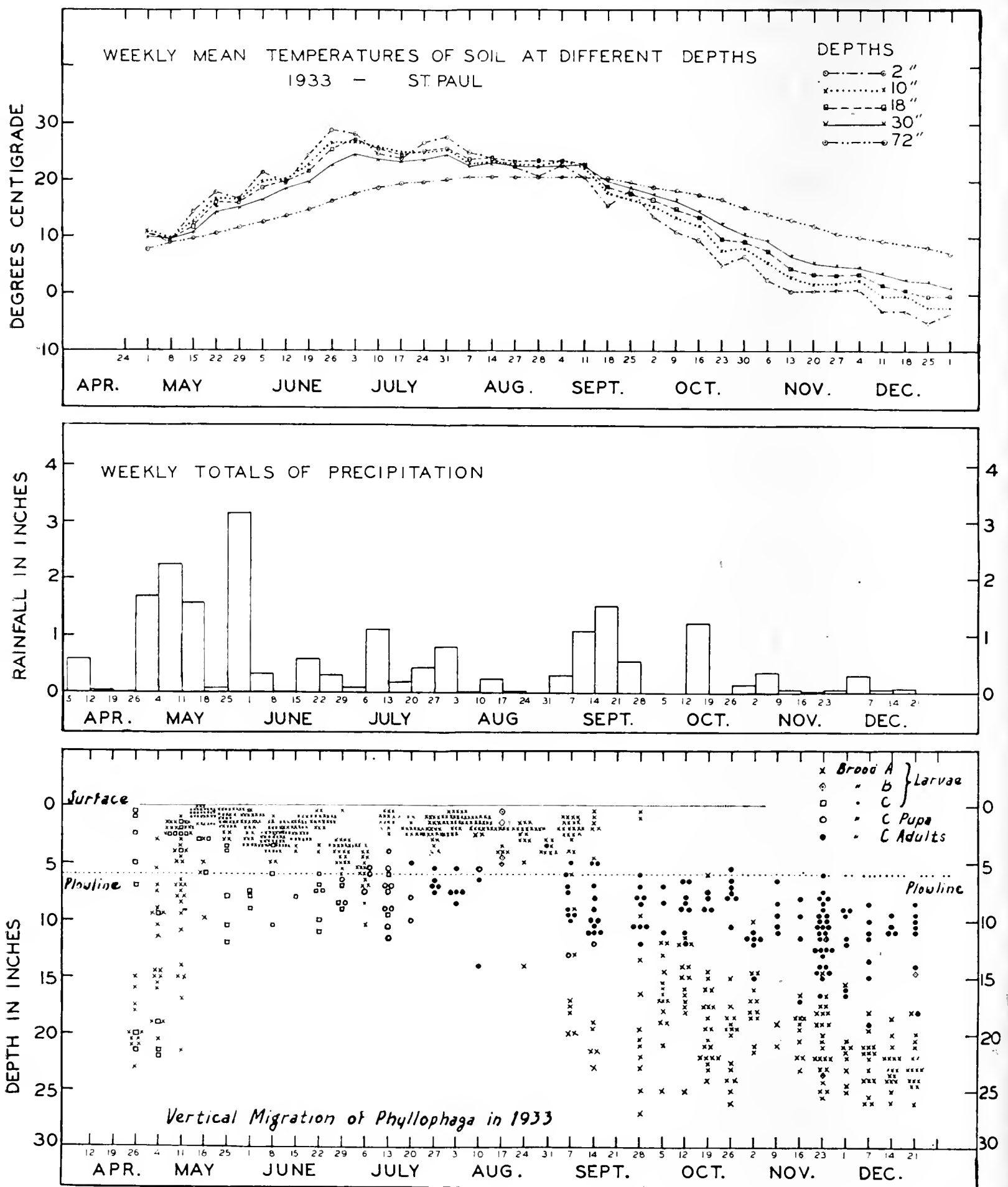


Fig. 1. Ecological studies on vertical movements in the life cycle of Phyllophaga.

grubs, in the third year of development, are first to appear near the surface of the ground, although usually remaining below the plow line, where they feed for a few weeks before pupation. They are joined in a course of a few days by the grubs of the second year of development which usually feed one or two inches below the surface.

This upward movement is conditioned likewise by spring rains and the rising soil temperatures, which are accompanied by the thawing of the frozen upper crust of soil. As moisture percolates downward, it softens the ground and moisture conditions become more favorable for the grubs in the upper layers of soil than in the general area of hibernation. This, together with the spring thermocline overturn, which takes place in the latter part of April, actually stimulates the grubs as well as the adult June beetles to move upward. Their ascent is accomplished with remarkable rapidity. Within a week or two all of the grubs have reached the upper layers of soil. Usually they are above the plow line, unless the upper soil is too dry for their normal activities as in the very dry summer of 1934 (Fig. 2).



The manner in which the grubs move upward or downward in the soil was extensively observed both in field and laboratory. The locomotion process of grubs was studied during the periodic excavations and in two-inch-diameter glass tubes, as well as in glass cages of one inch width filled with compact soil in the laboratory. During these studies kodachrome film recordings were made of their unique performance. It was found that the grub moves by a rather quick succession of somersaulting motions, paving its way by occasional lateral movements of the head, and evidently aided by the curved position of the grub's body. In time the cavity of the channel becomes firmly packed by successive somersaulting motion. The grubs can move downward at a much greater rate than they are able to move upward.

Once reaching the upper two or three inches of soil, the white grubs in the second year of growth continue to feed there until fall, if the periodic precipitation is normal and the soil not too dry. The grubs in the third year of development feed somewhat deeper, and

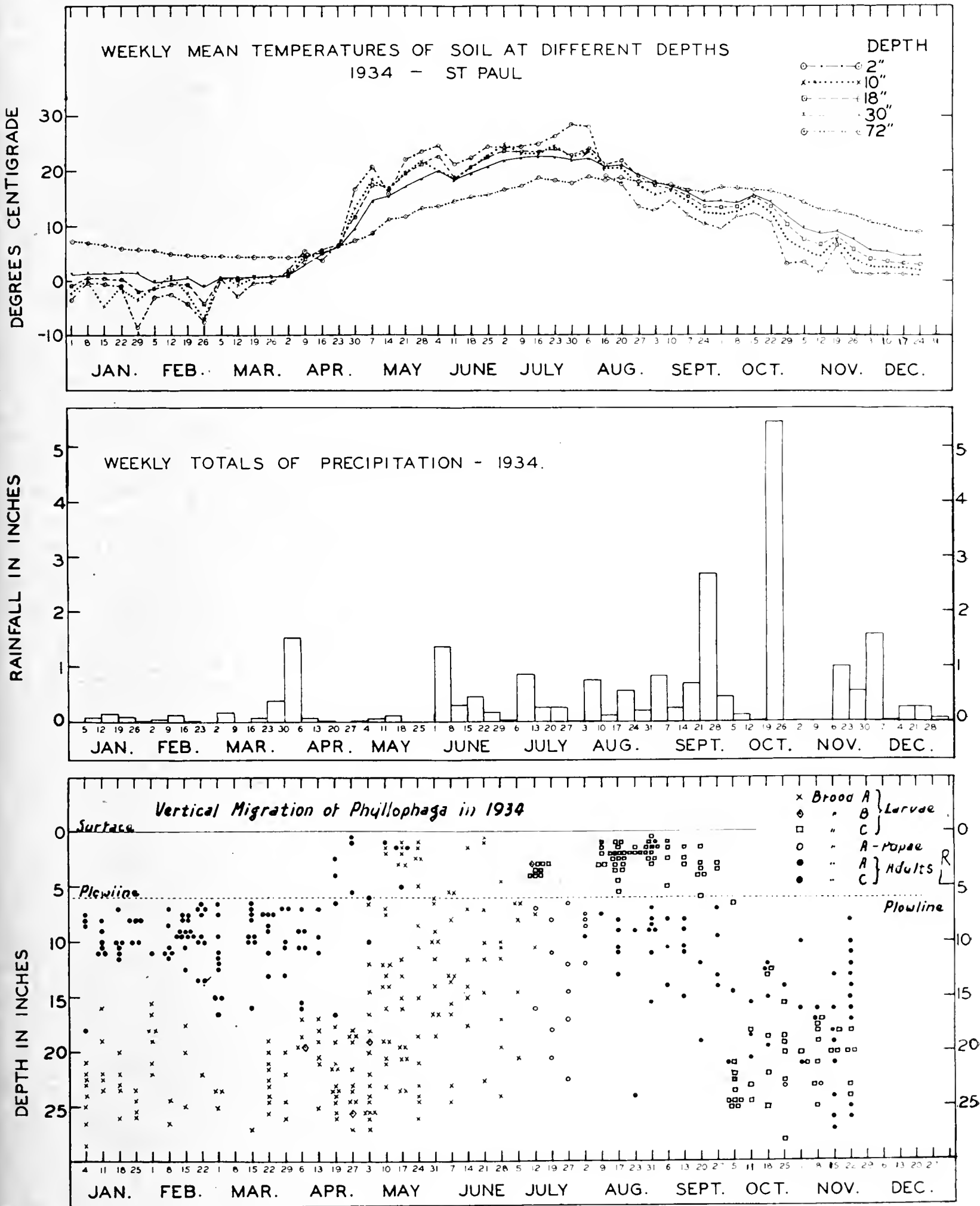


Fig. 2. Ecological studies on vertical movements in the life cycle of *Phyllophaga*.

usually below the plow line. It was determined that the grubs in their active feeding stages can tolerate a relatively low soil humidity. They apparently obtain sufficient moisture for their physiological development in the succulent roots of plants and thus depend less on the moisture condition of the surrounding soil.

From the soil moisture content as determined at various depths at the time of excavations, it is evident that grubs can feed in soil with only about 5 to 6 percent of moisture, or even less. However, they prefer higher percentages of soil moisture and quickly respond to small differences of soil moisture after each precipitation and move upward or downward to reach suitable conditions. The preferred soil moisture indicated for feeding stages of grubs is between 11 to 16 per cent, i.e. midpoint between the hygroscopic coefficient and moisture equivalent of a given soil.

In examining the levels at which the grubs feed it was found that, regardless of age or stage of development, they are remarkably sensitive to soil moisture. It will suffice to compare the graphs of weekly digging and weekly precipitation, for the years 1933 and 1934 (Figs. 1, 2), to detect the downward movement of grubs in dry periods of the summer in order to adjust themselves to preferred soil moisture conditions. The younger the grubs the more sensitive they are to deficiency of soil moisture, which results in gradual desiccation especially of the newly hatched grubs and up to their first molt. It was also observed that under dry soil conditions many eggs collapse and are unable to hatch, or the newly hatching grubs are unable to free themselves from the corion of the egg and finally perish by desiccation. On account of this drought effect on the young grubs in corresponding broods for the year, there was observed a marked reduction of grub populations of those broods with vulnerable stages of larvae. Even the reversal of economic importance of the broods was observed as conditioned by drought.

The drought effect on the depth of feeding and pupation of grubs can be best illustrated by the graph for 1934 (Fig. 2). Note that there was hardly any feeding above the plow line during that year, and most of the grubs were distributed down to 25 inches below the surface during the midsummer months.

Continuous studies of white grubs since 1932 to date, revealed that the order of importance of various broods in Minnesota, normally was "A", "C" and "B". Various less important species of *Phyllophaga* are definitely localized in given geographic areas of the state with apparent relation to the availability of plant host for the adult beetles and in some places brood "B" and "C" may be of greater importance than brood "A". Normally, however, brood "A" was of the greatest importance.

On the basis of the intensive and extensive studies of vertical migration of *Phyllophaga* and substantiated by the light trap records of June beetles, it is evident that there occurred a noticeable reduction of beetles and of grubs in brood "A" through the period of several three-year cycles during the drought years. At the same time the climatic conditions, especially the precipitation, favored the increase of population in brood "C". This resulted in the reversal of the importance of *Phyllophaga* broods. This buildup of brood "C" is especially evident through the years from 1938 to 1940 (Fig. 3). In actual agricultural practice, brood "C" became much more important than brood "A" in many sections of the state where timely precipitation favored the hatching of young larvae, and where correspondingly deficiency of soil moisture at vulnerable stages of grub development tremendously reduced the numerical population of brood "A".

In order to determine the effect of soil temperature and soil moisture on the development and the survival of grubs, a series of experiments were conducted in the laboratory. These experiments consisted of duplicate soil tanks maintained at constant temperatures and soil moistures. Six different temperatures ranging from the air temperature of the soil and at 16, 20, 24, 32 and 50° C. were kept at constant levels. The soil tanks were filled with the same field soil as was found in the area of the periodic diggings. This had a moisture equivalent of 18.6, a moisture holding capacity of 50.3, and pH of 7.35 being about neutral with a slight effervescence with dilute acid. All soil tanks were planted with wheat for grubs to feed on. Two soil tanks of each of the above mentioned temperature series were placed in four different series of moisture conditions as follows:

- (1) 6.86 percent soil moisture, being the hygroscopic coefficient of this soil.
- (2) 12.73 percent, the midpoint between hygroscopic coefficient and moisture equivalent.

- (3) 18.6 percent, the moisture equivalent of this soil, being about optimum for plant growth.
- (4) 34.44 percent, the midpoint between moisture equivalent and moisture holding capacity (saturation) of this soil.

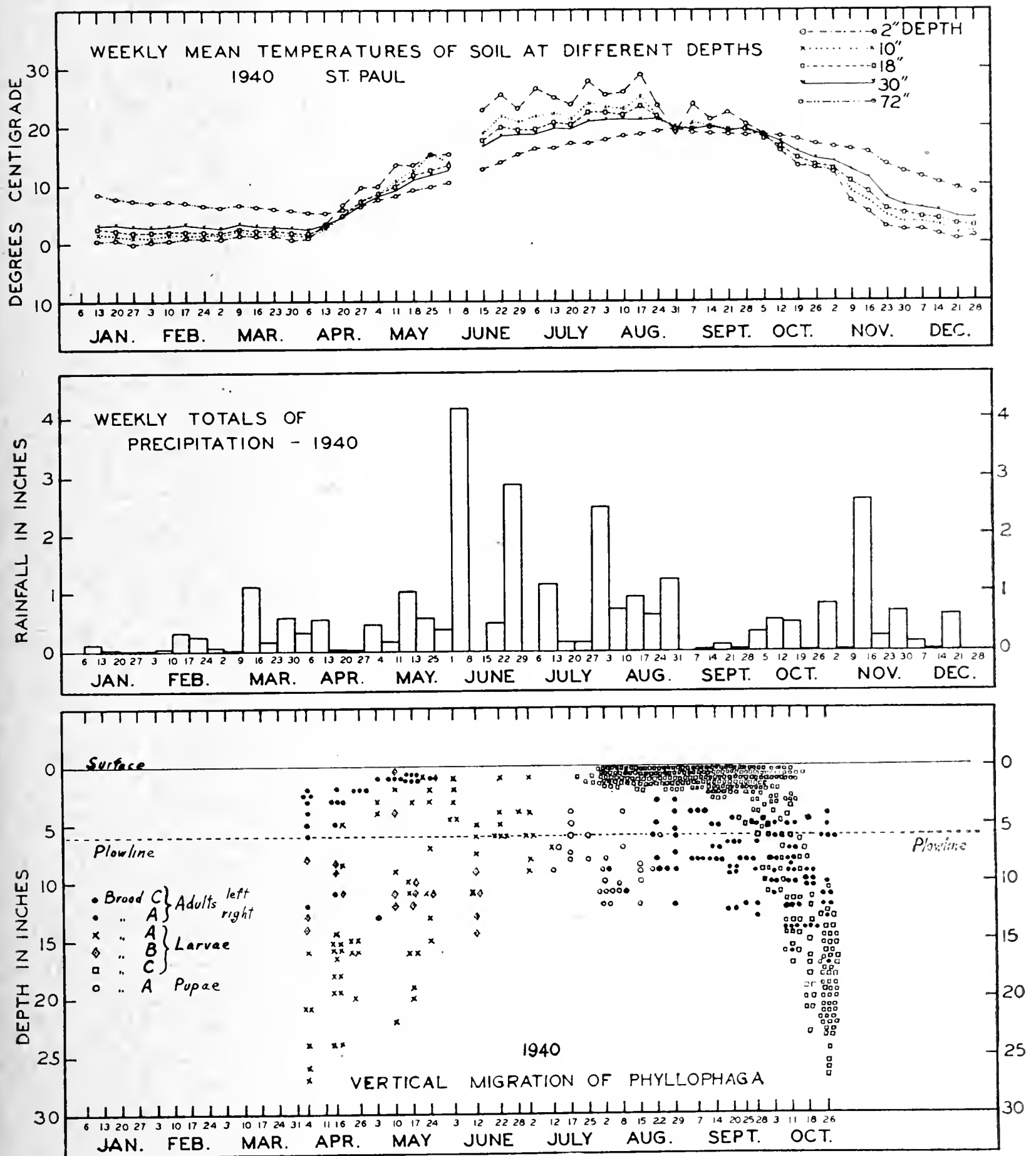


Fig. 3. Ecological studies on vertical movements in the life cycle of *Phyllophaga*.

After the constant temperature and moisture were stabilized, each tank was planted with ten recently hatched young grubs of *Phyllophaga fusca*. After one month of constant temperature and moisture treatments, the tanks were examined for surviving grubs.

Except at the two higher levels, soil temperature had relatively little effect on the survival of young grubs (see Table I). At these higher temperatures survival was greater with high percentage of soil moisture. At lower temperatures the grubs had a much better survival opportunity. It is quite evident that soil moisture plays a much more important role than temperature, and that young grubs have best survival opportunity in the soil maintained at the moisture equivalent level of 18.6 percent, which is the optimum soil moisture condition for plant growth in this soil. The next best moisture condition was

TABLE I. Number of Young Grubs Surviving After one Month in Tanks of Soil held at Constant Temperature and Moisture.

Temperature	Soil Moisture*, Percent				Total	
	6.86	12.73	18.6	34.44		
Air	4 2	7 3	4 4	6 2	21 11	32
16° C	4 0	6 5	6 6	6 4	22 15	
20° C	4 5	6 4	6 4	7 0	23 13	36
24° C	3 0	2 2	6 4	3 2	14 8	
32° C	0 0	1 0	1 0	4 0	6 0	6
50° C	0 0	0 0	0 0	0 0	0 0	
Total	22	36	41	34		133

\*These series represent, for the soil used in this experiment, respectively:—(1) the hygroscopic coefficient, (2) the midpoint between hygroscopic coefficient and moisture equivalent, (3) the moisture equivalent, and (4) the midpoint between moisture equivalent and moisture holding capacity.

found to be the midpoint between the hygroscopic coefficient and moisture equivalent for this soil, at 12.73 percent of soil moisture content. This was followed by the midpoint between saturation and moisture equivalent at 34.44 percent for the soil used. The least desirable ecological situation, so far as moisture is concerned, was the condition characterized by the hygroscopic coefficient of the soil which was equal to 6.86 percent for the soil used in the experiment.

It is indeed of interest to point out that the grubs in selecting their preferred levels for feeding or for other biological activities instinctively seek the soil moisture levels between the hygroscopic coefficient and the moisture equivalent of the soil. Naturally, under field conditions the soil moisture content is conditioned by the periodic precipitation, and the grubs are more likely to be found *at the levels of the midpoint between hygroscopic coefficient and the moisture equivalent*. This has been substantiated by the actual records of grubs' positions and the corresponding soil moisture determinations.

As was mentioned above, the grubs in their third year of development ascend from the mid part of April and before those that are in the second year of growth; the latter usually ascend in the first part of May. While the latter normally feed right under the surface of the soil, the former, as a rule, remain near the plow line, especially if they are more fully physiologically mature. Only the less fully developed grubs in the third year of development feed for any length of time close to the surface. Accumulated data indicate that after a few weeks of feeding, they begin in the latter part of June and in the first week of July to move downward, usually to some distance below the plow line, for pupation. The level of pupation is normally distributed between 5 and 13 inches and down to 18 inches in the presence of sufficient soil moisture. In the years of dry soil, as in 1934 (Fig. 2), many pupae were found as deep as 20 to 23 inches below the surface, and in relatively wet years (1939–1940) (Fig. 3), as shallow as 3 inches below the surface. The drier the upper level of soil, the deeper they move for pupation. The soil moisture determinations show that they are pupating at about 9 to 13 percent soil moisture content, which is about the midpoint between the hygroscopic coefficient and moisture equivalent of the soil.

Pupation usually extends from about the latter part of June through the first or second week of August; most of them pupate in July. Only rarely in the St. Paul area the pupae are found as late as the end of August.

Adults begin to emerge about July 20, but the majority emerge through August. Upon emergence they remain for some weeks in the earthen cells made by the pupating grubs, but there is sufficient evidence to show that with the advent of cold temperature the adult beetles of *Ph. fusca* and *Ph. rugosa* leave the cells in which they emerged and move downward to more suitable situations of temperature and moisture conditions. During the winter months the majority of adults of these species are found between the levels of plow line and 20 inches, and some as deep as 28 inches below the surface of the ground, evidently selecting the most suitable places in which to spend winter with reference to soil temperature and moisture conditions. In examining the records of soil temperatures during the winter months, it will be found that with the exception of 1934, soil temperature at 2 inches below the surface seldom went below 0° C. Thus, most of the grubs and the adults were situated between 0° C. and 5° C. above zero, although they can withstand winter temperature considerably below zero.

The adults of *Phyllophaga fusca* and *Ph. rugosa* remain in their hibernacula deep in the soil until early spring. There is evidence that the adults of *Ph. drakei* may overwinter only a few inches below the surface.

The spring ascent of the adult June beetles starts before any of the grubs begin to move upward. Records show that they move toward the surface before the spring soil thermocline, soon after the beginning of the rise of spring temperature and the thawing of the upper surface of the ground, which is accompanied by free water percolation into deeper layers. Soil moisture determinations show that the soil at that time, about the area of adult hibernation, becomes saturated, quite muddy, and unsuitable for the beetles. Usually they begin to move upward about the latter part of March or in the first half of April, depending on the season, and in a few days are all found only an inch or two below the surface, preparing to fly for mating and oviposition. The June beetle flight, in any numbers, seldom begins in April, usually in the second and third week of May, depending on the prevailing spring weather and especially the temperature. During the nocturnal flights, beetles mate and the oviposition takes place through the latter part of May and the entire month of June. The new grubs usually begin to hatch in the middle part of July and into the first part of August.





# Longevity and Oviposition of Moths of the Army Cutworm, *Chorizagrotis auxiliaris* (Grote) (Lepidoptera: Noctuidae), at Various Temperatures

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## ABSTRACT<sup>1</sup>

Moths of the army cutworm, *Chorizagrotis auxiliaris* (Grote), emerge in June. After a period of apparent aestivation during the summer they resume activity and lay eggs in late August and throughout September. In the laboratory, methods were devised to rear field-collected larvae to adults and investigations were conducted on longevity and oviposition at constant temperatures of 10°, 15°, 20°, 25°, and 30° C. for varying periods. Experimental series consisted of three replicates, each with 4 males and 5 females in a pint oviposition cage containing sifted soil to a depth of one-half inch and a vial of honey-water solution for food. The durations of the pre-oviposition period at 10°, 15°, 20°, and 25° C. were 150, 32, 16, and 25 days, respectively. When moths were placed at 10° C. for 20, 40, and 60 days and then moved to 25° C., the preoviposition periods at the latter temperature were 8, 7, and 5 days, respectively. Egg-laying extended over a period of 30 to 70 days and was of the shortest duration at 20° C. The mean durations of adult life at 25° and 15° C. were 11 and 23 days, respectively, for unfed moths and 50 and 57 days, respectively, for moths that had continuous access to food. After 180 days at 10° C., the mortality of fed moths was less than 50 per cent. The egg-laying capacity of *C. auxiliaris* exceeds that of many other Noctuidae. At various constant temperatures 75 females laid 62,270 eggs, averaging about 837 eggs per female. Several groups of five females laid from 8,000 to 9,000 eggs.

<sup>1</sup> The full text will be submitted to the *Journal of Economic Entomology*.



# Some Aspects of the Biology of the Diamondback Moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae), in Eastern Ontario

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## ABSTRACT<sup>1</sup>

The biology of the diamondback moth, *Plutella maculipennis* (Curt.), a pest of cruciferous crops in Ontario, was studied over a six-year period near the Central Experimental Farm, Ottawa.

The adults are inactive during the daytime and are found on the host plant. Activity begins just before dusk, reaching a peak well before midnight. Mating occurs on the day of emergence. Outdoors, caged females laid 18 to 356 eggs, the average being 159.4. Egg-laying began on the day of emergence and lasted about 10 days, the peak occurring on the first night of oviposition except when temperatures at sunset were below 66° F.

In the field, the number of eggs laid on individual cabbage plants ranged from 0 to 164. The ratio of those laid on the upper and lower leaf surfaces was approximately 3 to 2; few were laid on the stems and leaf petioles. Almost as many eggs were laid in small groups as were laid singly. In a field study cage the incubation period for 2,236 eggs ranged from 4 to 8 days, averaging 5.6.

The larvae feed from the lower leaf surface, consuming all the tissues except the veins and the upper epidermis. First-instar larvae, however, mine the leaf, feeding in the spongy mesophyll layer. For 2,022 larvae, the average durations of the instars, in days, were as follows: first, 4.5; second, 4.0; third, 4.1; and fourth, 4.9.

Pupation normally occurs on the host plant. The prepupal period was 1 to 2 days; for 2,000 individuals, the duration of the pupal stage was 5 to 15 days, the average being 8.5. The sex ratio was normal. The lifespan of 101 female moths averaged 16.2 days; that of 105 males 12.1 days.

There were four to six generations per year. The first generation developed mainly on cruciferous weeds; however, subsequent generations preferred to breed on cultivated Cruciferae. The insect apparently does not hibernate at Ottawa.

Studies with a controlled-interval light trap showed that the moth was in flight for an average of 136 days each year. The period of maximum flight was from late July to early September. The peak of flight in the night as determined by more than 11,000 captures occurred during the hourly period beginning 90 minutes after sunset. Both sexes were taken in progressively decreasing numbers throughout the night.

*Barbarea vulgaris* R. Br. is a preferred weed host. However, in an experiment on attractiveness of hosts, the insect showed little preference between the following cultivated crucifers: cabbage, cauliflower, kohlrabi, kale, Brussels sprouts, broccoli, and collards.

In the laboratory, temperature was shown to have a profound effect on the rate of development. The mean threshold of development value for the three immature stages was 45.3°F., and 510.4 day degrees were needed for the development of a complete generation.

<sup>1</sup> Article in part published. See: Harcourt, D. G. 1957. Biology of the diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera Plutellidae), in eastern Ontario. II. Life-history: behaviour, and host relationships. *Canadian Ent.* 89: 554-564.





# The History of the European Corn Borer, *Pyrausta nubilalis* (Hbn.) (Lepidoptera: Pyralidae), in Canada<sup>1</sup>

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## ABSTRACT

The European corn borer has been known to exist in Canada since 1920, where it was first discovered in southern Ontario. Since then it has spread throughout the corn-growing regions of the country, and is now found from the Maritime Provinces to south-central Saskatchewan. The corn borer has shown that it is adapted to a wide range of climate. In Ontario, the borer has become predominantly multivoltine after being univoltine for over 20 years; this change is most prominent in the southwestern peninsula. Multivoltinism is gradually extending northward; in favourable seasons a partial second generation occurs at Ottawa, Ont., and at Brandon, Man.

The shelled corn industry in Ontario suffered severe losses from the corn borer when only open-pollinated corn was grown, but stalk breakage has been reduced as a result of the development of hybrid corn. However, in recent years ear drop has become more noticeable owing to second-generation feeding. Increased corn acreages in the Maritimes and Manitoba make the corn borer an important pest in those provinces. In Ontario and Quebec, losses are minimized by a program of insecticide control. The development of corn varieties resistant to the insect holds promise.

## INTRODUCTION

The European corn borer is a native of Europe where it is a serious pest of corn, or maize, broom corn, hops, millet and hemp, and it is found wherever these crops are grown. According to Caffrey and Worthley (1927) corn, *Zea mays*, is injured to a greater extent than any other cultivated host, although there are many references in the literature dating back to 1835 of damage to other crops.

It is not known definitely how the corn borer first arrived in Canada but circumstantial evidence indicates that it obtained entrance, between 1910 and 1920, in shipments of broom corn from Austria (Spencer and Crawford, 1923). At that time the Plant Protection Division was not in operation and plants and plant products were not examined for possible noxious pests. A similar situation occurred in the United States, which imported quantities of broom corn from Hungary and Italy about the same time (Vinal, 1917). The corn borer was reported from Boston, Mass., in 1917 (loc. cit.), and from Welland, Ont., on August 10, 1920 (Caesar, 1928). The borer, however, was known to corn growers in the St. Thomas-Port Stanley, Ont., area as early as 1910 (McLaine, 1922). It was not reported to government officers, however, until 1920. It is possible that the infestation near Welland resulted from importing fresh table corn from New York State.

## EARLY HISTORY OF THE CORN BORER

The borer spread rapidly throughout the corn-growing areas of Ontario from 1920 to 1928. According to Caesar (1928), it became one of the most common themes of conversation in almost every farm home. Infestation was greatest in the counties of Essex and Kent, where the bulk of Canadian grain corn is grown. Within a period of six years, corn acreage in these two counties was reduced by 75 per cent. Many farmers seriously considered growing crops other than corn, but so extreme a measure was made unnecessary by a combination of circumstances.

In 1926 the Ontario Corn Borer Act was first put into force, and in 1927 this measure was extended. This Act required farmers in the main corn-growing areas of the province to suitably dispose of corn stubble in the spring, and so destroy overwintering borers. Various methods were adopted by corn growers in complying with the Act. Some farmers simply raked the stubble into windrows and destroyed the refuse by burning it. Other growers plowed the stubble under, and laboriously hand-picked the stubble brought to the surface through cultivation. Some growers in outlying districts simply ignored the

<sup>1</sup> Contribution No. 3593, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

Act and, annually, their fields were potential sources of infestation. Co-operation, in general, during the early years when the Act was in force was good, and there was reason for officials and growers to feel that this measure was responsible for reducing the borer population. In retrospect it is evident that this means of control was not as effective as thought, and that other factors were mainly responsible for the low infestation found after the initial upsurge in the mid 1920's.

Both Flint *et al.* (1931) and Davis (1935) have drawn attention to the great influence exerted by drought in delaying the spread of the corn borer in the Middle West. Davis stated that the season of 1934 definitely set back the borer several years. Stirrett (1938) showed that high temperature and low precipitation during June and July were associated with low infestation by the borer. There seems little reason to doubt that the principal factor in the reduction of the borer population in the early 1930's was the weather.

In a previous publication Wressell (1952) pointed out that larger acreages of hybrid corn were planted in the province from 1938 on. To what degree hybrid corn helped reduce borer infestation is not known. But the plant has a high degree of tolerance against the borer and stalk breakage, even in a year of severe infestation such as 1949, is greatly reduced.

In Eastern Canada, according to Keenan (1927), the borer was first found in Quebec in 1926, in several widely-scattered places. Generally speaking, corn is not so important a crop as in Ontario, but during the years following its first appearance it caused serious damage in sweet corn, and was especially prevalent in the vicinity of Montreal where table corn and canning corn are grown.

Gorham *et al.* (1929) reported that the borer was first found in the Maritimes in the St. John valley in 1928. The infestation was light and scattered, and from 1931 to 1944 the borer was not reported in New Brunswick. In 1945 the borer again appeared in that province probably the result, according to Gorham (1946), of a flight of moths from New England. It now appears to be an established pest in the Grand Lake-St. John region where sweet corn is an important truck crop.

The corn borer has been a pest of sweet corn in Nova Scotia since 1929 when it was first reported by Gilliatt (1929) as appearing in several widely-scattered areas. Within recent years the borer has caused serious damage to high-quality sweet corn grown in the upper Annapolis Valley (Fox and Neary, 1956).

The only known record of the presence of the corn borer on Prince Edward Island is that reported by Cannon (1950), who stated that two larvae were found on the Island in 1949.

In Western Canada the borer was recorded for the first time in both Saskatchewan and Manitoba in 1949 (Smith, 1950), presumably arriving there by way of the adjacent states of Minnesota and North Dakota. In Saskatchewan the first infestations were found in the semi-arid prairie area near Estevan, where sweet corn is grown in back-yard gardens as a table delicacy. By 1953 the borer had advanced as far north as Saskatoon (Stewart and Putnam, 1953), and in 1955 the western boundary of infestation was 50 miles west of the Third Meridian (McDonald, 1956). The infestation in 1955 in both Manitoba and Saskatchewan was the most severe recorded in those provinces.

### CHANGES IN THE STATUS OF THE CORN BORER

In New England the corn borer has always been multivoltine in habit, but in Ontario and the adjacent Lake States the borer was at first mainly univoltine; a trace of a second generation was present some seasons. Significant changes took place in the life-history of the borer about 1941, particularly in southwestern Ontario. The percentage of multivoltine borers increased so that by 1955 this strain was predominant in the husking corn area of the province. Wishart (1943) pointed out that the increase of the multivoltine strain in the borer population was gradual, but consistent in the earlier years. Wressell (1952), using the flight of moths to a light trap as a criterion, showed that in 1944 the greater percentage of borers belonged to the multivoltine strain in the grain corn area. Although there is less evidence of multivoltinism in the more northern corn-growing areas of Ontario, summer pupae have been found in the Ottawa district. It would appear that the corn borer population in Ontario is a complex of multiple and single generations, with

the multivoltine strain predominant in the southwestern sections; it occurs in other areas only in seasons favourable to its development.

In western Canada the same complex of characters is found, but to a lesser extent. The borer, however, has not been present long enough for an equilibrium to have been reached, and it is not possible to state with certainty whether the population will be univoltine or bivoltine. In the Maritimes, for example, bivoltinism was present when the borer first appeared in 1929, but there is no recent record of summer pupation.

Working with corn borers from the Harrow district in southwestern Ontario, the author has been able to demonstrate that multivoltinism is well established in that area. Larvae collected in the late fall have been exposed to a 38°F. temperature for two to three months to satisfy diapause conditions. They were then placed in an incubator set at a constant temperature of 85°F. and 65 to 75 per cent R. H. Four successive generations have been reared under these conditions with no apparent loss of vigour when fed on green string beans. During the first two generations, there was no evidence of diapause, but about 3 per cent of the larvae entered diapause during the third and fourth generations (Wressell, 1956).

Lees (1956) has suggested that both heredity and environment are concerned in the determinations of voltinism in the corn borer. This is in keeping with Arbuthnot's theory (1949) that both corn borer strains may be present in a region, but the less favoured strain may have reached the region ahead of the one best suited to survive in the environment. In time equilibrium is reached, and the strain best suited to the region will predominate.

## METHODS OF CONTROL

### BIOLOGICAL CONTROL

Two methods of biological control have been used in Canada in attempts to reduce corn borer populations by biological control. These methods are (a) dissemination of fungous spores to infested plants, (b) release of parasites in areas of high corn borer concentration. The results of both types of control have been reported elsewhere, and only brief summaries will be given here.

(a) Steinhaus (1949) has pointed out that entomogenous fungi in nature cause a regular and tremendous mortality of many insect pests in many parts of the world. Using the fungous *Beauveria bassiana* (Bals.), Stirrett *et al.* (1937) in 1936, and Beall *et al.* (1939) in 1938 demonstrated that effective control in the field by this means was possible. In 1938 up to 70 per cent control was obtained. It was found that time of application rather than rate of application was important, which is in keeping with the principles of insecticide control of the corn borer. This method of control no doubt would have been investigated further, but about this time the use of insecticides on corn was coming rapidly to the fore. This latter type of treatment proved to be much more effective, as well as easier to apply.

(b) According to Wishart (1943) the work with parasites of the corn borer in Canada was begun in 1923. During the following 20 years, nearly 5 million adults of 17 distinct species and two additional races of parasites were liberated in Ontario, Quebec, New Brunswick and Nova Scotia. Wishart stated that "no evidence appeared until 1940 to indicate a natural build-up of any species". Good initial establishment was followed by a rapidly diminishing population. By 1940, however, it was found that *Lydella* sp. was present in plentiful numbers along the Detroit river and near the shores of Lake Erie where a certain type of marsh existed. However, about this time the multivoltine strain of borer was beginning to increase, and as Wishart points out (*loc. cit.*) the establishment of the parasites of the corn borer has met with more success in areas like New England where the multiple generation occurs. The writer has made extensive field surveys of the larval population for the purpose of finding the annual infestation in southwestern Ontario each fall. In years when the multivoltine strain was plentiful, it has been noted that empty pupal cases of a dipterous parasite, presumably *Lydella* sp., although not plentiful, are often seen. It is possible that this species of parasite might be associated with the multivoltine strain of borer rather than with a particular environment. It is known that multivoltinism first made its appearance at LaSalle, on the Detroit river, and it is here that *Lydella* sp. has been found in numbers.

## INSECTICIDE CONTROL

The first successful use of insecticides against the corn borer in Canada was reported by Stirrett and Thompson (1941) in 1941. Previous tests made at Chatham in 1931 gave poor results, mainly because the insecticides available at that time were inadequate. In 1937 working in Connecticut, Batchelder *et al.* (1937) demonstrated that both derris and dual-fixed nicotine offered possibilities in the control of the corn borer. Baker and Questel (1939) at Toledo, Ohio, confirmed these results, although there was disagreement as to which insecticide was superior. Stirrett and Thompson (*loc. cit.*) used both derris and cryolite and found that the former was a superior insecticide and that cryolite, while it gave adequate control, had serious phytotoxic effects when used on corn. Wressell (1944) tested derris against fixed nicotine for use on canning corn and found that derris was much superior. For several years derris was the recommended insecticide in Canada for corn borer control. It was not, however, used to any great extent during the period 1940–1946 because war conditions caused this product to be in short supply. Also, growers were not cognizant of this method of control.

The discovery of DDT revolutionized the control of the corn borer by insecticides and proved to be of inestimable value to table corn and canning corn growers. Batchelder and Questel (1945) working at Toledo, Ohio, demonstrated the effectiveness of DDT in 1945. About the same time, Pepper and Carruth (1945) showed that the plant insecticide ryania was useful against the corn borer. Both these insecticides, together with several other newer hydrocarbon insecticides, were tested in Ontario by Wressell (1948) in 1947, and in New Brunswick by Pond (1949) in 1948. DDT and ryania are now recognized as the standard insecticides for the control of the corn borer in Canada (Wressell, 1954). DDT is the preferred insecticide because of availability, cheapness and ease of handling; emulsions, wettable powders and dusts are all recommended. However, when corn stover is fed to livestock, ryania is the approved insecticide. This material is of plant origin and is innocuous to warm-blooded animals. When DDT is used, on the other hand, a small amount is deposited in the body fat and/or milk of animals that feed on corn stover treated with this material.

More recently it has been shown by Cox *et al.* (1956) that granulated insecticides have a place in corn borer control, and a number of these materials are presently under study at the Chatham, Ont., laboratory. During the summer of 1956, it was evident that granular forms of endrin, heptachlor and DDT showed great promise for corn borer control in both sweet corn and grain corn in Canada.

Painter (1951) has pointed out that the general problems and possibilities of using corn varieties resistant to the corn borer were recognized very early. The development of hybrid corn intensified this type of research in recent years. The early information in Canada was obtained from varieties grown in corn-performance test plots. These varieties were subjected only to natural corn borer infestation. Resistance was not readily evident, but it was apparent that most hybrid corn varieties were remarkably tolerant to borer attack (Thompson, 1941). The growing of hybrid corn in the grain corn belt of Ontario has undoubtedly helped to reduce borer damage, but it is difficult to assess the rightful place of this means of control. In the meantime, a study of both Canadian and United States inbred varieties is being continued with a view to finding lines that are suitable for the work of the plant breeder, plant pathologist and entomologist.

For several years the writer has conducted studies, in various parts of Ontario, on the possible resistance of corn varieties. Replicated plots have been maintained on the Harrow Experimental Farm, and smaller plots have been under study at the Central Experimental Farm, Ottawa. At both places the plants were subjected only to natural infestation. At the Chatham Entomology Laboratory, both inbreds and single crosses have been hand-infested with a given number of corn borer eggs. A number of varieties have shown marked susceptibility, but a few inbreds show promise. It has been shown that certain inbreds are not attractive to the female moth for oviposition, but when infested with eggs such an inbred might be susceptible. Another problem that is being investigated is the resistance offered by certain inbreds to the first-generation borer and the susceptibility of the same inbred to the second-generation borer. This further complicates an already complex problem.



## PRESENT STATUS OF THE BORER IN CANADA

The corn borer remains a pest of prime importance in both Ontario and Quebec, but the methods of holding this insect in check are much better organized and more complete than formerly. Hybrid corn, the use of insecticides, and a more thorough understanding of the insect by the corn grower are all factors in reducing crop loss. With the knowledge now available about this insect, it seems improbable that outbreaks comparable with 1926-1927 will again occur. However, there is always the possibility that the corn borer may build resistance to present-day insecticides, although so far there has been no evidence of this. Within recent years, too, the increase of the multivoltine strain of borer in Ontario has brought important changes in the borer situation. Under favourable conditions second-generation borers feed in the region of the developing ear and may cause "ear drop" by boring into the ear shank. This type of injury, occurring in a year when stalk rot is prevalent such as happened in 1955, can cause serious damage in the grain corn district. Furthermore, the multivoltine strain has brought new developments in the canning corn industry in both Ontario and Quebec. Formerly, when the single-generation strain was predominant, late planting of canning corn was regarded as the best means of holding the borer in check in this important crop. This method of control is no longer effective, and the application of insecticides is now a standard procedure by the canning companies.

The borer is more important than formerly in the Maritime Provinces. Sweet corn acreages have been increased, and infestation by the borer has become more evident in recent years. The product, especially in the Wolfville-Kentville area of Nova Scotia, is a very superior type and the methods of grading and packaging for market are in keeping with the standards set by these provinces. Consequently, the sweet corn must be borer-free and greater attention is now paid to treating with insecticides, although the methods used are not as well organized as in the Central Provinces.

As pointed out earlier, the corn borer has spread widely throughout southern Saskatchewan, and it is well established in Manitoba. Corn in western Canada is mainly confined to back-yard gardens, but as such it is a highly regarded table delicacy. In Manitoba there is a considerable acreage of both grain corn and canning corn, and in recent years the borer has assumed a place of importance where these crops are grown. Because of the dearth of corn, however, some apprehension is felt because of the presence of the borer. This insect can and will attack peppers, potatoes and oats, as well as other crops in Ontario. Millet has been infested in Manitoba but, so far, there have been no reports of cereals being attacked in the Canadian West. As pointed out in an earlier publication (Wressell, 1953), the corn borer readily survives the cold temperatures and semi-arid conditions of the southern prairie regions. Winter temperatures here are comparable with those found at Orel, Russia, which is the northern limit for this insect in Europe.

So far the corn borer has not appeared in the province of Alberta. It has, however, spread well across the Great Plains in the United States, being present in both Montana and Wyoming (Shotwell, 1954). Canning corn is grown in considerable acreage on irrigated land in the Taber-Lethbridge district of southern Alberta. Conditions here are favourable for corn borer attack, and it is probably only a matter of time before this insect appears in the area.

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# Entomology in the Pacific Area with Special Reference to Agriculture

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Entomologists in the Pacific area, particularly on the outer Pacific islands, are faced with a number of special problems peculiar to the region. The entomologists are unequally distributed, and some large political or geographical areas, consisting of very many islands, or of large territories which are very little known, have far too few entomologists assigned to them. In many parts of the Pacific the economic level of agriculture is such that much expenditure for entomological research or chemical control appears to the administrators or growers to be unjustified. The inhabitants of a large portion of the Pacific islands exist on a level of subsistence agriculture, involving from very few to a moderate number of plants, mainly native to the general region. Generally there are only one or two crops which are grown for export purposes.

The most important of these crops is the coconut palm, and for many areas copra is the only, or at least the chief, export or cash crop. The copra market is such that coconut cultivation depends on a minimum of labor expenditure and other investment, so that general use of insecticides is rarely warranted from the standpoint of the growers. In several areas, as a result of pest damage, or the effects of war, coconut groves or plantations have been abandoned, or superficially harvested without being adequately cleaned up. In some areas where *Oryctes rhinoceros* has done great damage considerable government expenditure has been necessary to bring areas back under copra production to permit the population to be self-supporting again, or to prevent the spread of the beetle to more important copra producing areas.

Another aspect of Pacific island entomology is that relatively few of the well-known temperate pests are important, and often the local pests are native insects, or those introduced from nearby tropical areas. With the greater diversification of agriculture, native insects are constantly appearing as pests of newly established crops, particularly on the continental islands, though rarely so on the more isolated oceanic islands. Likewise with the increase of air transport, more and more pests are being distributed to new areas. On the oceanic islands this can be of the greatest danger, for with absence of potential parasites, predators and competitors, insects establishing themselves in a disharmonic fauna with abundance of favorable food, can do damage of a serious nature. For the same reasons, of course, introductions of appropriate parasites or predators may sometimes prove highly successful. However, the search for, and testing of, the beneficial insects for biological control is very expensive, and the lesser developed areas have to depend on those with greater specialization in entomology, associated with higher standard of living, for help in this regard.

The insect fauna of the Pacific area is very unequally known, and for the major portion it is insufficiently known. Thus not only are many native pests not readily identifiable, but of many of the species very little is known of the general habits and ecology. Furthermore, not enough is known regarding many of the pests introduced from neighboring tropical areas.

Hawaii enjoys a high standard of agriculture centering around sugar cane and pineapple, and secondarily, papaya and passion fruit export, with other crops, and cattle, grown for local consumption, though much of the food consumed is imported from California. Biological control, quarantine, and fruit-fly control concern a considerable proportion of the many entomologists in Hawaii. Since the early days of modern agriculture in Hawaii, most of the sugar cane and pineapple pests have been kept out, or brought under effective biological control. Some of the striking successes were with the sugar cane leafhopper (*Perkinsiella saccharicida* by *Cyrtorhinus*), scarab grubs feeding on cane roots (*Anomala orientalis* by *Campsomeris annulata*) and sugar cane weevil by *Microceromasia* (tachinid fly). Fruit flies are still a serious problem, but much progress has been made, as is being reported in another paper at this Congress, by L. D. Christenson.

Several weed plants invading grazing land, introduced from tropical America, are receiving considerable attention. Mr. N. L. H. Krauss, parasite explorer for the Territory, has been introducing moths, cerambycid beetles and flies, to control *Lantana camara*, *Eupatorium glandulosum* and *Schinus terebenthifolius*, some of the pest plants. A number of scale insects, white flies and aphids attack fruits and ornamentals. The giant African snail is also the concern of entomologists, and two predacious snails and a predacious beetle have been introduced to attack it. The principal organizations concerned with agricultural entomology in Hawaii are the Agriculture Experiment Station of the University of Hawaii, the Territorial Board of Agriculture and Forestry, the U.S.D.A. Fruitfly Laboratory, the U.S.D.A. Plant Quarantine Branch, the Hawaiian Sugar Planters' Association Experiment Station, and the Pineapple Research Institute.

Micronesia has many introduced pests but many of them attack crops cultivated to a rather limited extent. The principal pest of coconut is *Oryctes rhinoceros*, which did very great damage in Palau, and which has been combatted for several years now by an expensive clean-up campaign to eliminate larval breeding habitats, as well as by biological control efforts. Other coconut pests include three native species of *Brontispa* (hispine beetles), one of which caused much damage after introduction from the Carolines to the Marianas; the long and short-horned grasshoppers, the coconut scale, *Aspidiotus destructor*, and the red coconut scale, *Furcaspis oceanica*. Other pests include species of *Icerya* on breadfruit, fulgoroid leafhoppers on taro, borers in cacao, common pests of maize, banana and sugar cane weevils, several aphids and aleyrodids, and others. There is now only one agricultural entomologist in Micronesia.

Samoa has fairly limited insect problems, centering around stick insects and *Agonoxena* moths on coconut; *Prodenia litura*, thrips and hoppers on taro; *Adoretus* beetles on cacao; *Icerya* on breadfruit; scab moth (*Lamprosema*) on banana; and a few others. *Scolia ruficornis* was established for *Oryctes* control 10 years ago. The South Pacific Commission maintained a New Zealand entomologist in Western Samoa for two years until recently doing experiments on *Oryctes* control. In recent years there has been an entomologist in American Samoa.

Tahiti has most of the pests found in Samoa, except for *Oryctes* and *Adoretus*.

The Cook Islands have in the main the same pests as Tahiti.

Tonga has approximately the same pests as found in Samoa, including *Oryctes*, which is only in the Vavao group, and was introduced in 1951. There are no resident entomologists in Tahiti, Cook Islands, or Tonga.

Fiji, like Hawaii, has been the scene of some of the most remarkable successes in biological control. These include the control of the coconut hispine, *Promecotheca*, by the Javan wasp, *Pleurotropis parvulus*; the coconut scale, *Aspidiotus destructor*, by the coccinellid, *Cryptognatha nodiceps*, and other predators and parasites; the coconut moth, *Levuana iridescens*, by the fly, *Ptychomyia remota*; and the weed, *Clidemia hirta*, by the thrips, *Liothrips urichi*. The most serious current pest is *Oryctes rhinoceros*, which was introduced to Viti Levu in 1952 or 1953, probably from Samoa. About £50,000 per year is being spent on control, involving crews climbing palms and inserting a BHC-sawdust mixture in petiole-bases, and similarly treating, or burning or removing compost or other larval habitats. The aim is to keep the *Oryctes* population down until more effective natural enemies can be established, and at the same time to prevent the beetle spreading to the other islands of the Fiji group, which are more important in copra production than Viti Levu. Another current pest in Fiji is the banana scab moth, *Lamprosema*. The Fiji government is about to employ a retired entomologist to search for parasites of the moth in New Guinea. Others include fulgoroid hoppers on sugar cane; a small rhinoceros beetle in roots of cane; leaf hoppers and moths on rice; scale insects on citrus and other fruits; noctuid moths and other widespread pests. There is one entomologist in Fiji, besides a supervisor of the *Oryctes* control campaign.

New Caledonia has an endemic hispine beetle as well as *Brontispa longissima* in coconut; thrips, noctuid moths, hoppers and weevils in sugar cane; *Prodenia* on maize; leaf beetles on maize, wheat, and vegetables; hoppers on rice; a sphinx moth on sweet potato; aphids and moths on taro; *Gnorimoschema operculella* on tobacco, tomato and potato; fruit flies in papaya; scales and aphids on citrus, and others. There is one agricultural

entomologist at Noumea who also serves the other French colonies in Oceania. Also, Noumea is the headquarters of the South Pacific Commission, which employs an entomologist (plant and animal quarantine officer). Also an Indian entomologist is now employed in searching for natural enemies of *Oryctes rhinoceros* in Asia; and an insect pathologist, on a Rockefeller grant, is searching for *Oryctes* diseases in Asia.

The New Hebrides has the hispines, *Promecotheca opacicollis* and *Brontispa longissima*, the small coconut weevil, *Oryctes centaurus* (possibly a misidentification), the pentatomid *Axiagastus*, the moths, *Tirathaba* and *Agonoxena*, and phasmids on coconut. Various scales attack citrus; cane weevil and hoppers feed on sugar cane; *Lamprosema* and banana weevil on banana; chrysomelids, coreids and a pyralid on cucurbits; hoppers on taro; a tortricid, noctuid, mealybug, hoppers, and an *Elytrurus* weevil on cacao; and *Epilachna* on tomato.

The Solomon Islands have coconut nutfall caused by coreid bugs of the genus *Amblypelta*, which are indirectly encouraged by presence of the ant *Iridomyrmex*. Other coconut pests are rhinoceros beetles of the genera *Trichogomphus*, *Scapanes* and *Papuana*, *Rhynchophorus* palm weevils, the hispine, *Brontispa longissima* and others, and phasmids, grasshoppers, moths and scales. Other pests include weevils in banana and sugar cane; grasshoppers, *Leptocorisa* bugs and *Papuana* on rice; *Amblypelta* on cassava; *Epilachna* on tobacco and tomato; *Heliothis* on tobacco; sphingids and tortoise beetles on sweet potato; galerucids on cucurbits; and *Papuana* and sphingids on taro.

New Guinea and the Bismarcks have their entomological problems centering around pests of coconut, cacao, coffee and sweet potato. Rubber is relatively free from insect attack, except at the bud-stock stage, when some cerambycids and weevils attack it. Coconut pests include long-horned grasshoppers of genera *Segestes*, *Sexava* and some new genera; rhinoceros beetles including *Oryctes rhinoceros* in New Britain, New Ireland and Netherlands New Guinea; *Oryctes centaurus* in Papua; several species of *Scapanes*, *Papuana*, *Oryctoderus* and others, *Rhynchophorus* palm weevils, several hispine beetles, particularly *Promecotheca papuana* (on which I am speaking in another section of this Congress), and *Brontispa longissima*. Cacao pests include capsid bugs of several genera, which cause dropping of the pods; several kinds of weevils, particularly *Pantorhytes*, in both larval and adult stages; and several cerambycid borers and eumolpid leaf beetles, besides limacodid moths and others. Coffee pests are girdling and defoliating weevils, of new genera; a leaf-rolling moth, and others being discussed in another paper by Dr. Szent-Ivany. Sweet potato pests include the sphinx, *Herse convolvuli*, the tortoise beetle, *Aspidomorpha testudinaria*, and the *Formicomimus* sweet potato weevil. Various new highland insects are attacking European vegetables as they are introduced. *Leptoglossus australis*, the coreid, attacks passion fruit; *Papuana* rhinoceros beetles damage taro; and white grubs destroy turf on airstrips in the highlands. The fauna of New Guinea is so rich and so little known that as new crops are introduced, they are attacked by native insects which often prove to represent new species or new genera. Doubtless as the insect fauna and its ecology become better known, some beneficial insects may be found which might be introduced to advantage to other Pacific islands.

Australia, with its different fauna and climate, has many pests and problems differing from those of other parts of the Pacific, though North Queensland shares many types of insects with New Guinea. Here sugar cane is important, and is attacked by dynastid and melolonthid grubs feeding on the roots, hoppers and leaf beetles. Also in Queensland weed plants are being fought with insects. Probably the most outstanding success of weed-control by insects was that of the *Opuntia* cactus by the moth *Cactoblastis*. Another weed, St. John's wort, was controlled by leaf beetles from Europe. Now lantana moths from Central America are being introduced to Queensland from Hawaii. The major agricultural undertaking in Australia is grazing, and important pests in this field are locusts, pasture grubs, sheep blow flies and termites. In southern and eastern Australia citrus and other fruit tree scale insects are important as well as fruit flies. Other pests include *Heliothis armigera* on maize; the dicky rice weevil, *Mauleterpes spiniceps*, boring in citrus; and a noctuid moth which pierces citrus fruit.

New Zealand, again, has problems peculiar to it, being different faunally and climatically from other parts of the Pacific. The most important insects are pasture pests, particularly the melolonthid, *Costelytra zealandica*, and the hepialid, *Oxycanus despectus*. Recently



great success has been attained in combatting these by incorporating BHC or DDT with the superphosphate topdressing, and for the first time the grassland farmer has become a large scale user of insecticides. More localized pasture pests are the armyworms, *Persectania aversa* and *Pseudalectia separata*, which will also probably come under better control from the above treatments. Better control of sheep maggot is being attained through use of dieldrin. There is an attempt to secure parasites for biological control of *Coleophora* sp. attacking white clover seed. The most important farm crops are crucifers and while control of *Pieris rapae* by *Pteromalus* and *Apanteles* is adequate, the status of the parasites introduced for *Plutella* is being investigated. A new pest is the apple leaf midge, *Dasyneura mali*. The cixiid, *Oliarus atkinsoni*, was incriminated as vector of "yellow leaf" disease of the native fibre plant, *Phormium tenax*. Much attention is being given to forest insects as a result of establishment of pulp mills to utilize the introduced pines. Biological control with *Ibolia* is being attempted on *Sirex*. Native moths such as *Selidoxema suavis* are appearing as defoliators. The quarantine situation has much improved in recent years, as regards legislation, staffing and facilities.

Indonesia, being an area of extremely rich fauna, with a great variety of native and introduced crops, has a large number of native pests, and many widespread south Asian species. A few of the more important pests are *Oryctes rhinoceros*, *Rhynchophorus* weevils, *Sexava* long-horned grasshoppers, various moths and hispine beetles on coconut; moth borers, hoppers, and locusts on rice; rhinoceros beetles, borers, hoppers, white flies, and locusts on sugar cane; and many borers, moths, and scale insects on tropical fruits.

The Philippines likewise have numerous pests, many of them the same as or similar to those in Indonesia. Among the more serious pests are locusts, rice-boring moths, hoppers on cane and rice, *Oryctes rhinoceros*, palm weevils and hispines.

Taiwan has problems relating mainly to sugar cane, rice, fruit and vegetable culture. Important pests are moth borers and defoliators, leaf beetles, hoppers, scale insects, sweet potato weevils, banana weevils and cane weevils.

The Ryukyu Islands suffer mainly from depredations of *Euscepes* and *Formicomimus* weevils and leaf beetles on sweet potato; borers, hoppers and aleyrodids on sugar cane; leaf beetles and caterpillars on vegetables; and borers and scales on citrus and other fruit trees.

Japan, with its highly diversified agriculture, has innumerable entomological problems, with countless native and introduced pests. Some of the important ones are boring moths, locusts, leaf beetles and hoppers on rice; defoliators on sweet potato; borers and earworms in maize; longicorn borers in citrus, mulberry, fig and chestnut; pea weevils, various fruit moths, nut weevils and defoliators. The many experiment stations in Japan have contributed much on the ecology and control of many of these pests. Insecticides are being used on a large scale.

In conclusion, our knowledge of the insects of the Pacific area is very uneven and much basic work remains to be done. Increased facilities of transport are bringing the various scattered parts of the Pacific into closer communication and cooperation, but at the same time pests are being further spread by the same means. This emphasizes the very great importance of quarantines, and the need for greater cooperation for mutual protection from pests.

An organization concerned with the exchange of ideas and cooperation among Pacific countries is the Pacific Science Association. It maintains standing committees, one of which is Entomology. It has a permanent secretariat, located at Bishop Museum in Honolulu, which issues news summaries at frequent intervals, and it organizes the Pacific Science Congresses, held every three or four years. The Ninth Congress is to be at Bangkok late in 1957, when symposia will be held on various phases of Pacific entomology.

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<sup>1</sup> Additional data and citations may be found in these references.



# The Smaller Rice Leaf-Miner, *Hydrellia griseola* Fallén, in Japan

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## ABSTRACT

The smaller rice leaf-miner, *Hydrellia griseola* Fallén, has been known since 1933 as one of the serious rice pests in Japan, especially in the north temperate regions such as Hokkaido and the Tohoku district of Honshu.

Life history studies of this insect indicate that it may produce five to eight generations per year, of which the second generation may occur on the rice plant and the other generations on wild grasses. In Hokkaido, the adult flies begin to emerge at the end of April or early in May from the hibernated puparia. Though the duration of each stage is affected by climatic and other environmental factors, the length of time from laying of the eggs to emergence of the adults is about one month, consisting of about a week in the egg stage, and about 2 weeks each in the larval and pupal stages. The life span of the adult female ranges from 5 to 18 days, during which time 20 to 30 eggs are deposited on leaf blades lying on the water surface. Due to the longevity of the adult, a two-week overlap in generations can be expected. This insect is adaptable to a fairly low temperature environment, possessing a wide zone of lower critical temperatures. Its host range numbers 46 plant species belonging to 32 genera of 4 families in Hokkaido. Gramineae predominate, but Monocotyledonous plants are the object of feeding and pupation mines, while Dicotyledons are the object of pupation mines only. In Japan the insect is attacked by 14 Hymenopterous parasites belonging to 5 families. Of these *Opius* sp. and *Chorebus* sp. are widely distributed and abundant in number; *Hemiteles* sp. is presumably a secondary parasite.

Since its discovery in 1933 this insect has several times severely infested the paddy fields of Japan, the 1954 outbreak being the most severe. The outbreaks, however, were sporadic. According to our investigations, severe outbreaks may be associated with climatic conditions in the current and preceding years, in combination with environmental factors of paddy fields or the growth phase of the rice plants. Low temperatures in the preceding summer result in an increase in numbers in the fall season, and higher temperatures than usual during winter, accompanied by an early thaw, contribute to a low mortality of the increased populations. Relatively high temperatures in early spring and low temperatures from May to July provide favorable conditions for the development and increase of this pest. Moreover, synchronism between the oviposition period, the transplanting of rice seedlings, deep water in the paddy fields, and slow, weak growth of the plants because of low temperatures increase the damage. Such a combination of unfavorable conditions does not ordinarily occur in any single season, but in 1954 almost all of these factors were present facilitating a severe outbreak.

Of the recommended control measures, spraying or dusting with insecticides is the most effective known at present. Early dusting with BHC 1 to 3% gamma isomer against adults and newly hatched larvae is fairly effective, but against the growing larvae, a parathion 1.5% dust or a dieldrin emulsion is more satisfactory. Of course, control may be achieved by a combination of the application of insecticides and rational cultural practices, including water management and the transplanting of vigorous seedlings in an optimum period.

## INTRODUCTION

In Japan, two species of leaf-mining Diptera have been known as the insect pests of the rice plant. They are the rice leaf-miner (*Agromyza oryzae* Munakata) and the smaller rice leaf-miner (*Hydrellia griseola* Fallén), the former being distributed in the north temperate region of Japan and in Siberia, and the latter being widely distributed over Europe, Siberia, the northern part of China, Japan and the Pacific coast of North America. The rice leaf-miner has been referred to as one of the most destructive pests of the rice plant in Japan for more than fifty years, causing noticeable injury every year, though variable in yearly prevalence (Kuwayama, 1950). The first evidence of the occurrence of the smaller rice leaf-miner in Japan was obtained in 1933 by the writer, who discovered rice plants injured by this pest in the paddy field of the Wakkanai Experimental Farm, a branch of the Hokkaido Agricultural Experiment Station, which is located in the northernmost part of

Japan (Kuwayama, 1954). The rice plant had not been cultivated in that area before that year. Since then injuries by this pest have often been reported from Hokkaido and the northeastern and middle parts of Honshu, and in 1938 and 1942 were also reported from Tanegashima, the southernmost island of Japan. However, the 1954 outbreak although sporadic was the worst reported in Japan. The infested paddy fields were estimated to total over 300,000 hectares in Hokkaido and 17 prefectures of northern Honshu. In the case of Hokkaido that year this pest spread over 104,700 hectares. It damaged 75,000 hectares, representing 54.7% of Hokkaido's paddy rice acreage, and reduced the crop by 39,600 kiloliters, although 3,000 tons of BHC dust and 178 tons of parathion dust were used in control measures. It is noteworthy that in 1953 a great outbreak of this pest occurred in practically all of the rice growing areas of California, U.S.A., after a 30-year lapse since the previous severe attack (Lange, Ingebretsen, and Davis, 1953). On the occasion of the 1954 outbreak the writer and many other entomologists in Japan observed this leaf-miner ecologically and engaged in experiments on control measures; hence our knowledge of this pest was remarkably increased. Some noteworthy results of these studies were compiled by the writer and published as Special Report No. 3 from the Society of Plant Protection of North Japan in December, 1955 (Kuwayama, 1955). Some of the more important aspects of these studies are presented in this paper.

### SEASONAL HISTORY AND HABITS

According to the observations of Kreiter (1927) and Mesnil (1931) in Europe this insect has two generations per year, and Lange, Jr., *et al.* (1953) observed in California that there are at least three generations annually. However, according to the phenological observations made by Sakurai, Matsumoto and Tomioka in Hokkaido there are seven generations during the season, of which the second generation lives on the rice plant and the others primarily on wild grasses. They considered that this insect has at least five to six generations per year. Also Suzuki and Koyama in field studies during the season of 1954 in Akita Prefecture observed that there were seven generations and also presumed the probability of eight generations per year in view of the oviposition in the first decade of April. In Fukushima Prefecture, Endo and Kanno found this insect to have four or five generations on the Gramineous weeds each season. These facts coincide with the result of Tomioka's experiments on the influence of thermo-hygro conditions on the postembryonic development of this insect. He concluded by means of the law of total effective temperature that under the climatic conditions of Sapporo and its vicinity in 1954 this insect might have passed six generations between May and October, excepting the first generation originating from the hibernated puparia, adults of which emerged in the latter half of April, that is, the zone of lower critical temperature. Overwintering state is not uniform by localities. In Europe Wilke (1924) stated that this insect hibernates as puparia in soil. In Hokkaido it passes the winter in the pupal stage on the surface of swampy land or in shallow soil in empty ditches, but Suzuki and Koyama observed in Akita Prefecture that it overwintered as a larva on the food or shelter plants but that a small percentage were in the pupal stage. Omori and Oya observed in Iwate Prefecture that this insect continued its development through the winter in the warmer area where grasses keep green even in winter near springs or streams, and every stage was seen all the year round, but in an environment where the water dried up and grasses were blasted in winter, only larvae and puparia were observed. These facts indicate that diapause does not occur during the overwintering of this insect because it possesses a wide zone of lower critical temperatures. Based on the experiments by various research workers thermal responses of the adult flies and larvae of this insect are recorded in Table I.

The experiments were conducted under the gradually rising temperature at the rate of  $1^{\circ}\text{C}$  in every 3 or 4 minutes using adult flies and matured larvae. From the data tabulated, the optimum temperature zone of adult activity is in the range covering  $13^{\circ}\text{C}$  to  $30^{\circ}\text{C}$  and that of larvae from  $11^{\circ}\text{C}$  to  $32^{\circ}\text{C}$ ; so it seems that this insect is adaptable to a fairly low temperature environment.

The duration of each stage varies widely with the climatic and other environmental factors. According to observation made by Sakurai *et al.* in Hokkaido, the incubation period was 4 to 5 days under  $21^{\circ}\text{C}$ ; the larval period averaged 7.5 days under  $25^{\circ}\text{C}$ , 10.3 days under  $20^{\circ}\text{C}$ ; and 15.6 days under  $17^{\circ}\text{C}$ ; that is, it takes about 2 weeks under natural conditions during the latter part of June and the early part of July in Hokkaido; the pupal

period ranged from 6 to 10 days during middle and latter part of July (average room temperature  $19.4^{\circ}\text{C}$ ) and 10 to 20 days during late June to early July ( $16.0^{\circ}\text{C}$ ). Suzuki and Koyama from their experimental results tabulated the length of time for postembryonic development and the longevity of adult flies (Tables II and III).

The adults are essentially water-loving and fly close to the surface of water, sometimes skilfully walking on it. They prefer to rest upon the leaf blades, particularly those lying on the surface of water, and also prefer the leaves lying prone on the water for oviposition sites. From one to fifteen or more eggs may be laid on one blade, the maximum being 64 eggs. They are laid singly on the leaf blades and almost all are on the surface, although occasionally on the reverse side. When the larvae hatch, they immediately commence to mine the leaf blade and feed on the green cells, causing the leaf to shrivel and become a dirty ochreous or brown colour. These damaged leaves subsequently lie prostrate on the surface of the water. The mine is linear in shape, enlarging in length as well as width with the progress of development, but because two or more larvae mine ordinarily in one leaf blade, the leaf may wither as a result of the complete destruction of chlorophyll. Velocity of feeding or mining by larvae differs widely among individuals. The results of measuring mines every day after hatching, which may indicate the amount of feeding, are presented in Table IV.

The larvae often change mines in the course of their feeding and they pupate inside the leaf. Puparia are usually in the feeding mines, but frequently in a pupation mine newly made by matured larvae on the leaf blade of the host or any other plants.

Thus, the length of time from laying of the eggs to emergence of the adults from May to July is about a month in Hokkaido. Koyama and Suzuki estimated this length of time at about 27 days at  $18^{\circ}\text{C}$  average temperature. These facts coincide with observations made by Kreiter (1927) in Leningrad, U.S.S.R. The overlapping of the generations is observable and it is possible to obtain all stages of this insect during the season.

### HOST PLANTS

In Hokkaido, through field observations and experiments on host selection, Iwata and the writer recorded 46 species belonging to 32 genera of 4 families as hosts of this insect in 1954. Among these hosts Gramineaceous plants predominate, 41 species being enumerated. Monocotyledonous plants such as Gramineae, Cyperaceae and Alismataceae are oviposited by the adult flies and the leaves are injured by larval feeding, while Dicotyledons such as Leguminosae are merely the object of pupation mines. White clover (*Trifolium repens*), one of the latter, was found only in Hokkaido. Though the host selections of this insect differ widely according to environmental conditions, rice (*Oryza sativa*) and *Phalaris arundinacea* are most suitable as food plants, being followed by *Agrostis palustris*, Orchard grass (*Dactylis glomeratus*), *Glyceria leptolepis* and *Alopecurus pratensis*.

Okazaki and Itagaki also observed the host plants in Yamagata Prefecture. According to them, ovipositions are observable only on the Gramineae and Cyperaceae, such as the species of *Alopecurus*, *Poa*, *Anthoxanthum*, *Isachne*, *Carex*, *Phalaris*, *Calamagrostis* and *Bromus*.

According to field observations by Kawase in Ishikawa Prefecture in 1954, five species of Gramineae, including rice, barley and wheat, were the host plants of this insect.

As this leaf-miner has a polyphagous habit, additional host plants might be added in future in Japan.

### PARASITES AS NATURAL ENEMIES

In Europe *Coelinius hydrelliae* Kawall has been known as a parasite of this leaf-miner for a long time (Taschenberg, 1880). Yokoo (1936) reported from Korea *Dacnusa* sp. and *Biosteres* sp., the former attaining 19.3% in the rate of parasitism and the latter 10.0%. Nepveu and D'Aguilar in 1951 reported from France a Braconid, probably belonging to *Ademon*. According to Lange, Jr., et al. (1953), two Braconids, *Chorebus aquaticus* Muesebeck and *Opius hydrelliae* Muesebeck, are known as parasites of this leaf-miner in California, where they play a part in keeping down attacks of this pest in the years without heavy outbreak. Although no record of the parasites of this insect in Japan had been published, in 1954 there were found 14 species of Hymenopterous parasites belonging to 5 families from the puparia bred at the Hokkaido and Tohoku National Agricultural Experi-

TABLE I. Range of Mean Value of Temperature, at which Various Conditions of Activity are Shown.

Adult flies

Condition of activity	SUZUKI (Akita Pref.)	OMORI & OYA (Iwate Pref.)	TOMIOKA (Hokkaido)	TAKEUCHI (Hokkaido)
	°C	°C	°C	°C
Slight movement	1.7– 3.4	1.4– 2.0	4.3– 7.7	2.3– 6.0
Standing on feet	3.0– 4.5	2.3– 3.6	5.9– 9.2	4.0– 8.6
Walking	5.3–10.6	4.5– 7.2	8.7–12.1	10.6–12.2
First flying	14.5	14.1–16.6	15.7–19.1	19.8–21.2
Rest			27.4–29.5	
Nervous	29.2–30.4	31.0–35.5	34.4–36.5	29.4–32.0
Paralysis	35.5–38.2			36.3–39.9
Falling down	41.1–43.2	38.5–40.7	40.7–42.7	
Death	43.2–45.0	41.0–42.5	42.4–43.9	39.0–43.6

Larvae

Condition of activity	SUZUKI (Akita Pref.)	TOMIOKA (Hokkaido)	TAKEUCHI (Hokkaido)
	°C	°C	°C
Slight movement	3.4– 5.1	12.2–14.2	4.4
Crawling	9.9–13.3	14.2–15.1	10.7
Rest		30.4–32.3	
Nervous	30.9–34.5	33.2–34.2	31.5
Paralysis	35.5–39.0		
Falling down	38.9–41.7	41.8–42.9	40.7
Death	43.7–44.9	43.2–44.2	35.7

TABLE II. The Length of Time for Incubation, Larval and Pupal Stages.

Temperature (°C)			Egg	Larva	Pupa	Total*
Min.	Max.	Av.				
			days	days	days	days
14.6	26.0	18.06	6.54	16.01	10.32	32.93 ± 1.16
20.0	26.4	21.07	5.33	14.00	8.33	27.66 ± 2.49
25.0	27.5	25.30	4.50	–	–	–

\*Reliability 95%

TABLE III. Longevity of Adult Flies.

Average temperature (°C)	Food	Longevity of ♂	Longevity of ♀	Oviposition period	Total deposited eggs per one ♀
		days	days	days	
18.1	No	5.50 ± 2.05	5.25 ± 1.52	—	—
19.2	Supply with diluted honey	4.67 ± 0.73	18.00 ± 3.17	12.33 ± 2.49	31.00 ± 4.92

TABLE IV. Velocity of Mining by Larvae.

Items \ Days after hatching	0	1	2	3	4	5	6
	mm	mm	mm	mm	mm	mm	mm
Max.	10.0	14.0	21.0	28.0	40.0	69.0	85.0
Min.	0.5	3.0	7.0	12.0	22.0	40.0	78.0
Average	3.1	7.2	12.7	19.4	28.6	54.5	81.5
Number of individuals examined	14	14	14	13	9	2	2

TABLE V. Environmental Factors Influencing the Severe Outbreak in 1954.

Envir- on- men- tal factors \ Prefectures	Early trans- planting	Deep irrigation	Moun- tainous districts	Cool irrigation water	Sparse planting	Slow growth of plant	Weak growth of plant*
Hokkaido	o	o				o	o
Aomori	o						
Iwate	o	o			o	o	
Akita	o	o	o	o			o
Yamagata	o	o	o	o			o
Miyagi	o	o	o		o	o	o
Fukushima	o	o	o		o		
Niigata						o	o
Toyama			o			o	
Ishikawa	o						
Nagano	o		o				

\*Especially in the case of the leaves lying on the surface of water after transplanting.



ment Stations and also at the Hokkaido, Iwate, Miyagi, Fukushima, and Ishikawa Prefectural Agricultural Experiment Stations. They were as follows:

Braconidae:—*Chorebus* sp.; *Merites?* sp.; *Opius* sp.

Ichneumonidae:—*Hemiteles* sp.

Proctotrupidae:—*Ismarus* sp.

Pteromalidae:—*Trichomalus* sp.; *Merismus* sp.

Europhidae:—*Solenotus* sp.; *Neochrysocharis* sp.; *Rhopalotus* sp.; *Elachertus?* sp.; *Mestocharis?* sp.; *Paracrias* sp.; *Asecodes* sp.

Of these species, *Opius* sp. and *Chorebus* sp., followed by *Trichomalus* sp., are widely distributed and abundant, presumably playing a role in checking serious damage done by this leaf-miner. Two species of Braconidae are the same genera as those of the parasites in the U.S.A., but it has not yet been determined whether they are identical or not. *Hemiteles* sp. of Ichneumonidae is probably a secondary parasite.

### REASONS FOR SEVERE OUTBREAK

As stated before, the smaller rice leaf-miner has broken out several times in the paddy field of some districts in Japan, especially in the north temperate regions. The outbreaks, however, were sporadic and not continued year by year. This leaf-miner feeds on numerous wild grasses and does not come to the rice plant ordinarily during most years.

To determine the causes of outbreaks of this leaf-miner on rice, environmental factors were investigated in the paddy fields severely infested in the 1954 outbreak, and the climatic factors were analyzed in relation to the outbreak. The results of investigations on environmental factors are tabulated in Table V.

By the analysis of climatic factors, some facts become noteworthy: (1) air temperatures during the growing season in the year preceding severe outbreak, especially in July and August, were relatively low and duration of sunshine was short; (2) air temperatures during winter were remarkably high in the Tohoku and Hokuriku districts; (3) snow-melting in spring was rather earlier than usual in Hokkaido; and (4) air temperatures in the last 10 days of April of that year were high, but in May and later months were markedly low.

On account of the adaptability of this insect to fairly low temperature environment, the low temperatures during the summer of the year preceding an outbreak have favorable effect on the biotic potential of summer generations due to the escape from high temperature repression; accordingly the population density increases in the fall. Moreover, the high temperatures during winter or early snow-melting in spring contribute to the reduction of mortality.

The high temperatures in late April of that year accelerated the emergence of adult flies and these oviposited on wild grasses favorable for the buildup of this insect prior to the transplanting of rice plants. Early transplanting is synchronised with the next oviposition period, so that egg laying is localized on these rice plants. High water or deep irrigation just after transplanting results in the increase of leaves lying prone on the water, which are favorable for oviposition sites, and also high water encourages the development of the larvae. Furthermore, the slow and weak growth of the rice plants due to cool temperatures during May and July makes them more susceptible to the injury of this pest.

A severe outbreak of this leaf-miner, such as in 1954, may be associated with climatic conditions of that year and the preceding year in combination with environmental factors of paddy field, such as synchronism of oviposition and transplanting periods, deep irrigation, slow and weak growth of the rice plant and so on. Moreover, the worst injury in the 1954 outbreak was to some extent attributable to delayed detection by rice growers because of the long lapse between severe attacks.

### CONTROL MEASURES

As experimental results in 1950 indicated that BHC dust (.5% gamma isomer) was far superior to that of derris emulsion, in the 1954 outbreak BHC dust was recommended. It is effective against the adult flies and newly hatched larvae, but less effective against the growing larvae. So, later, the use of parathion dust was recommended. At the same time

many experiments were conducted on the insecticides. According to the experiments in Hokkaido by Endo, Nakamura, and Morikawa, against the adult flies, parathion 1.5% dust is most effective, being followed by BHC dust (1% gamma isomer) and Diazinon 1% dust; against eggs BHC dust (3% gamma isomer) is most effective, being followed by parathion 1.5% dust and BHC dust (1% gamma isomer), while Diazinon 1% dust is less effective. According to the experiments in Fukushima Prefecture by Endo and Kanno, against the 3rd instar larvae, 0.05% solution of parathion 46.6% emulsion resulted in complete control, being followed by 0.05% solution of BHC (10% gamma isomer) and 0.05% solution of DDT 20% emulsion.

On the contrary, Lange, Jr. *et al.* (1953) report that spraying with dieldrin or heptachlor at the rate of 0.5 pound of actual chemical per acre in 10 gallons of water applied by airplane brought good results in California on the occasion of the 1953 outbreak. In 1954 Koyama conducted experiments on new insecticides against this insect, in comparison with BHC in Akita Prefecture. According to the results he obtained, solutions of dieldrin, endrin, parathion, and diazinon emulsions were all more effective than BHC dust (1% gamma isomer). Spraying once with either 0.5% solution of dieldrin 18% emulsion or endrin 18.5% emulsion, 0.1% solution of parathion 46.6% emulsion, or spraying twice with either 0.25% solution of dieldrin or endrin emulsion, 0.17% solution of diazinon 17% emulsion may have satisfactory results against this pest. Though dieldrin was inferior in ovicidal effect, it showed almost perfect control because of the residual effect. Koyama concluded that spraying once with 0.5% solution of dieldrin 18% emulsion or spraying twice with 0.25% solution of the same at 10 days interval may be recommended in future outbreaks.

Control of the leaf-miner may be achieved by combining the application of effective insecticides with rational cultural practices, such as water management, especially lowering of the water in the paddy field, transplanting of vigorous seedlings in an optimum period and so on. As Lange, Jr. *et al.* observed in California, it is the writer's observation in Japan that weak plants in deep water were susceptible to decay or were not strong enough to force their way to the water surface.

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# The Prognosis of Occurrence of Noxious Insects

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In Czechoslovakia, phytopathological prognosis is being studied very carefully, in particular the problem of forecasting an epidemic occurrence of noxious insects. Regular lectures on this subject are delivered in our Universities and in 1955 the respective textbooks were published, which, as far as I know, comprised the first general elaboration of this problem. Recently, a new edition has been prepared for publication which also is to be translated into other languages.

Entomological prognosis is based on agrometeorology, bioclimatology, physiology, ecology, zoogeography, biocenology, biometrics, gradology and further biological branches of science as well as on mathematics, "Functions of Random Variables", on new measuring methods, and on other branches of science the knowledge of which I have tried to apply in practice.

Having elaborated the theoretical basis of prognosis I have set down general instructions of the practical prognostication of noxious insects; I have collected the most important methods to be applied and finally worked out the prognosis of certain important insect pests, especially sugar-beet pests.

Research in the prognosis of noxious insects, though only in its beginning, is becoming an increasingly more important aid to practical entomology.

Its importance may on the whole be summarized as follows:

1. Facilitation of preventive protection and prophylaxis, particularly the prognostication of the occurrence of forecasting an epidemic of noxious insects in time by following their gradology and relation to factors exerting influence upon their spreading.

2. Facilitation of actual therapeutic protection by estimating the future conditions and by selecting the most suitable protective means and measures.

3. Facilitation of the farmers' work by signalling the critical periods in which the pests should be sought for, information as to the symptoms of their occurrence, etc.

4. Securing production and timely distribution of requisites and insecticides to the most threatened areas to make immediate measures possible.

5. Elaboration of entomological statistics and prognosis enabling long-term planning—building of chemical factories, manufacture of mechanical means according to the prognosis of the occurrence of insects, etc.

6. Issuing of legal, especially quarantine provisions on pests, the greatest hazard of which should be estimated prior to their spreading and causing great damage.

7. Economical calculations of profitable protection of plants according to the prognosis both of future development and noxiousness of insects.

8. Distribution of more resistant sorts or varieties of plants according to the prognosis of pest occurrence.

9. Estimates of yield and quality of agricultural plants according to the prognosis of noxiousness of insects and consequent securing of raw materials for food, industry and export.

10. Selection of the manner of plant cultivation according to the prognosis of insect occurrence—modification of the crop rotation, preparation of the soil, choice of the fertilizers, fight against drought, time and manner of sowing, protection of plants during vegetation, weed killing, etc.

11. Organization of biological protection according to the prognosis of the occurrence of noxious insects—pasture breeding of poultry, breeding of useful birds, especially of partridges and pheasants, artificial cultivation of useful insects or entomophytous microorganisms, etc.

12. Laying of snares, traps, digging of ditches and similar measures according to the prognosis of pest occurrence.

13. Cultivation of plants to render them resistant to such pests as we foretell to be the most noxious in the years to come.

14. Foretelling the menace to plants by noxious insects when introducing new plants.

15. Determination of the trend of applied research, laying the basis of experiments, providing literature, etc., according to the prognosis in order to solve the most actual future problems.

In general, we endeavour to direct the prognosis so as to avoid as much damage as possible, i.e. thoroughly to study the pest and the manner of protection against it before its gradation reaches over-reproduction.

In practice we issue long term prognosis in the time of planning, a prognosis for the next vegetation period before the beginning of vegetation and short-term prognosis in the course of a year, if necessary.

A prognosis concerning each of the pests would require a separate survey. In general, we distinguish, on the one hand, a *scientific* prognosis, i.e. a research solution and a search for a suitable method of prognosis; on the other hand, a *practical* prognosis, in which case we compile the prognostication according to the very facts ascertained by the research work so as to be able to foresee the future occurrence of insects according to the momentary situation and factors acting simultaneously.

According to practical needs, we distinguish a *qualitative* prognosis "forecasting the kind of pest" and a *quantitative* one "number of pests". Moreover, we foretell the place of occurrence "*local* prognosis", the time of occurrence "*time* prognosis", the *noxiousness* of insects, *the menace* to plants, future damage, etc.

The above problems have been elaborated in the manuscript of a book on phytopathological prognosis, containing more than 500 pages, which will be published by the Czechoslovak Academy of Agricultural Science in Prague. The work comprises introductory chapters on the significance and tasks of prognosis and of its position in science; also a theoretical part deals with the influence of internal and external factors, food, carriers of diseases, enemies as well as of man upon the noxious agents.

The practical part contains the rudiments of entomological diagnosis, findings, classification, signal-service, reports, statistics, topography, cartography and evaluation of pest occurrence, all of which should be unified upon an international basis as is the case with meteorology. Quoted also are the most important biological, mathematical and other methods for diagnosing the pests, a survey of meteorological prognosis, phenology and their utilization in a prognosis. As in the case of the insect pests, diseases, weeds and abiotic factors have also been worked out.

The chapter on actual prognosis contains instructions regarding its application, a survey of organization of the prognosis, reports and a scheme of various types of prognosis as well as practical examples. The most important experience and results obtained hitherto in the prognostication of dangerous pests and diseases of the important cultural plants have been summed up in a special part.

I suggest that the next Entomological Congress, or its Committee, deal more fully with the problems of prognosis from the international point of view so that the suggested methods, the means of ascertainment, classification, signal-service and prognosis in entomology be unified and completed. I also suggest discussion on the proposal of phytopathological and entomological symbols which might facilitate international cooperation as is the case with meteorology, phenology, chemistry etc. I further suggest discussion on the problem of an international exchange of information concerning the spreading of dangerous pests all over the world. The Committee, which would discuss this problem, could make use of my hitherto gained results, since it is not possible, in the course of a short lecture, to deal with them in detail but only to call attention to them.

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# New Method of Forecast of the Rates of Plant-Lice Propagation

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## ABSTRACT<sup>1</sup>

Forecasting the mass propagation of insects is of great importance not only from theoretical but also from the practical point of view.

Mass propagation of organisms may take place only under favorable environmental conditions. The factors affecting the rate of insect propagation are very diversified, and their influence is far from being one and the same when they act in different combinations. These circumstances make it extremely difficult to determine the influence of environmental factors.

Since insects respond to changes in the environment, it is theoretically possible to determine morphological indices in the organism which reflect the response of the organism to the whole complex of environmental conditions.

With aphids, we have developed a very convenient index. Under favorable conditions aphids give rise to apterous forms; however, when conditions are deteriorating, the imaginal wing discs in the nymphs begin to develop. Survival is related to the accumulation of adipose tissue. The adipose cells are formed only in the young nymph from undifferentiated embryonic elements located in the area of the basal membrane of the connective tissue. This membrane underlies the hypodermis where the rudimentary wing buds or discs are found. Under unfavorable conditions these undifferentiated elements produce a cell material of another kind which is used for the development of the imaginal discs.

The development of alate forms in aphids is an adaptive phenomenon. Since it is caused by unfavorable conditions at a given site, and results in their migration to new sites, determination of the alation rate should allow us to forecast fluctuations in their numbers.

Data pertaining to *Aphis laburni* Kltnb. on white acacias, and *A. gossypii* Glov. in the cotton fields in Uzbekistan showed that if the percentage of nymphs with wing rudiments in aphid colonies does not exceed 25-30, the population builds up within the next 7-10 days. The lower the percentage of aphid nymphs with wing rudiments, the faster is the rate of the aphid population increase. When the percentage of aphid nymphs with wing rudiments reaches 40, the number of aphids remains at the same level for a week. If the percentage of larvae with wing rudiments exceeds 40-45, populations of *A. laburni*, which migrates faster, decrease in the next 7-10 days, whereas populations of *A. gossypii*, which is less mobile, decrease in the next 10-16 days.

The number of aphids is greatly influenced by predators and parasites. However, according to our observations and records, the aphid enemies are unable, without the interference of man, to exert their influence on the very character of the forthcoming change in the number of aphids, for a progressive increase and decrease of the population of natural enemies takes place only after a corresponding increase or decrease in the population of aphids.

Since conditions in different field plots are not identical, our samples were taken from different points. In the cotton fields of Central Asia it was found that for a field up to one hectare in size, collecting of 100 specimens from not less than 50 equidistant plants, while walking along the diagonal of the field was sufficient.

When collecting the aphids in the field it was necessary to place them in a fixing fluid at once; young nymphs may acquire wing rudiments within 12 hours.

The forecast of aphid populations depends not only upon the species, but upon climatic and other factors.

<sup>1</sup> To be published in *Jour. of General Biology*, Moscow, 5.



# Insects Affecting Banana Production in Central America

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## ABSTRACT

Red rust thrips, *Chaetanophothrips orchidii* (Moulton), is considered to be one of the most serious insect pests of bananas. This species has been known from the Far East for a number of years. In 1931 it was found in Panama and rapidly spread through Central America. Effects of control measures on the biotic balance within the banana plantation have been noted.

The banana root borer, *Cosmopolites sordidus* (Germ.), causes serious losses to the banana industry by reducing the weight of the stem of fruit, and is one of the main causes of fruit losses within the plantation due to fallen plants.

The banana stalk borer, *Castniomera humboldti* (Bois.), has been a minor pest of bananas for several years. Recently it has become a major pest in Costa Rica and Panama, where considerable fruit has been lost due to the activity of the larvae which feed within the stalk of the banana plant.

Minor pests of bananas are Florida red scale, *Chrysomphalus aonidum* (L.), and the coconut scale, *Aspidiotus destructor* Sign. These are generally controlled by the lady bird beetles *Pentilia castanea* Muls. and *Scymnus* sp. *Platynota rostrana* (Walker), the tortricid fruit caterpillar, is considered a minor pest but can become serious if precautions are not taken in the use of insecticides which tend to upset the biotic balance. The leaf-feeding caterpillar, *Ceramidia butleri* Moeschler is sporadic in occurrence and is generally controlled by the chalcid parasite *Trichogramma minutum* Riley. Other minor pests include the flower thrips *Frankliniella parvula* Hood throughout Central America and *F. musaeperda* Hood in the Dominican Republic. The chalcid wasp *Oraesema costaricensis* Wheeler & Wheeler, which is parasitic on ants, often damages banana fruit in oviposition. Several of these minor pests have become serious following the wide use of organic insecticides.

Insect control in relation to banana production in Central America is a relatively new field. The necessity of producing high quality fruit and of increasing production per acre to offset rising costs has placed considerably more emphasis on entomological research.

Until recent years, the control of insect pests on bananas was considered a minor problem. Fruit was produced cheaply and in quantity so that insect-damaged fruit could be discarded. The insect control then practiced was aimed at protecting the fruit by covering it with paper bags or by cultural control incorporated into the management of the plantations rather than at reducing the insect population by insecticidal treatment.

Three major insect pests of bananas found in Central America are red rust thrips, *Chaetanophothrips orchidii* (Moulton); banana root borer, *Cosmopolites sordidus* (Germ.); and banana stalk borer, *Castniomera humboldti* Bdv. The minor pests are Florida red scale, *Chrysomphalus aonidum* (L.); coconut scale, *Aspidiotus destructor* Sign.; the fruit-scarring caterpillar, *Platynota rostrana* (Walker); the leaf-feeding caterpillar, *Ceramidia butleri* Moeschler; and a flower thrips, *Frankliniella parvula* Hood.

Red rust thrips have been known for a number of years in the Far East. They were discussed by Caldwell (1938) and considered at that time as one of the limiting factors in the production of bananas in Australia. They were recognized by Johnson (1932) in Panama where the damage to bananas had been noted as early as 1926 but had not been associated with an insect. Deal (1937) worked out the life history and established control by means of bags applied to the stems of fruit. Flynn (1954) restudied the life history and found that each stage was approximately ten days in length.

The present name of the red rust thrips was established by Hood (1954). It was formerly known as *Anaphothrips signipennis* Bagnall (Johnson, 1932), and *Scirtothrips signipennis* (Bagnall) (Caldwell, 1938). There is still some taxonomic confusion concerning this thrips since there are two distinct types in Central America. On the Caribbean coast of Panama and Costa Rica, both sexes occur while on the Pacific coast of Panama and Costa Rica, on the north coast of Honduras, and on the east coast of Guatemala, only the par-

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thenogenic female is found. The former causes a much more severe blemish than does the latter type. There is a third thrips, as yet unidentified but apparently a distinct species, causing red rust of bananas in Ecuador.

The damage caused by the thrips is a reddish peel blemish usually found between the fingers of fruit. Slight damage does not detract from the appearance of the ripened fruit but more severe damage has a leathery appearance and greatly reduces the market value of the bananas.

In the late 1930s, red rust thrips control was established in the banana regions of Honduras by placing a paper bag over the hanging stem of fruit. This bag was applied when the fruit was from 10 to 14 days old and left on for a period of five weeks. Damage that occurred to the fruit after the bags were removed was not severe enough to affect the appearance.

After World War II, due to the increased cost of bags and of labor, a more economical method of control was sought. Experiments with the chlorinated hydrocarbon insecticides available at that time were started. Screening tests were set up to determine the effectiveness of the materials as ground sprays since the pupal stage of this thrips is passed in the soil. At that time it was not deemed feasible to apply insecticides to the fruit. DDT in both emulsifiable concentrate and wettable powder forms was first used as a ground spray at the rate of 2 pounds of toxicant per acre. Partial control was gained in areas of light infestations.

Later, fruit spraying was attempted with DDT and with Lindane. Lindane was effective but a slight off-flavor was noticeable in the mature fruit. DDT as a fruit spray gave fair results but when large areas were treated, heavy populations of Florida redscale appeared which detracted more from the appearance of the fruit than did the red rust.

DDT dust was applied by aircraft to a new plantation in Panama in an attempt to control thrips before the fruit emerged. This aerial dusting was effective in controlling the thrips on the pseudostem but had no apparent effect on those in the soil. In Panama, Brann (1954) applied dieldrin granules by hand to simulate aircraft dusting while in Honduras dieldrin was applied to the soil as a ground spray. Experimental results were excellent and field-scale trials, based on the results of these experiments, were initiated. Five-hundred-acre plots in Panama and in Costa Rica were treated with dieldrin granules applied by aircraft at the rate of 3 pounds toxicant per acre. The results were outstanding. After three weeks, thrips counts were down to zero in these areas. Later in 1954, thirty thousand acres in Costa Rica and 3,000 acres in Panama and in 1955, six thousand acres in Honduras were treated and the same results obtained. The only re-infestation noted to date is in one small area in Panama which might have been passed over during the application.

While these field trials were under way, experiments were established with dieldrin emulsifiable concentrate used to spray the hanging stem of fruit only. One quart of the concentrate containing 1.5 pounds of dieldrin per gallon in 100 gallons of water applied in a cycle of two sprays ten days apart provided excellent control.

The banana root borer, *Cosmopolites sordidus*, has been a pest of bananas for several years and is distributed throughout the banana zones of the tropical world. The life cycle has been reported by Frogatt (1926) from Australia, Moznette (1920) in Florida, and Pinto (1928) in Ceylon. In all of these life history studies, the habits of the insect are similar: the eggs are laid at the base of the pseudostem or on the rhizome; the larvae bore into the rhizome, forming tunnels which become larger as the insect develops and may completely destroy the rhizome; pupation takes place in the larval tunnel, usually near the outer surface of the rhizome. The entire development takes about 40 days under Central American conditions.

The feeding of the larvae within the rhizome damages the plant which may produce a small, light-weight stem of fruit or, in many cases, causes the plant to topple over without producing a stem of fruit. Plants weakened by the borer are easily blown over during periods of light winds with complete loss of plant and fruit. When borer-infested seed bits are planted, the resulting plant is small and requires more time to become established and to mature a stem of fruit. Careful inspection of seed before planting will eliminate replanting of large areas later.

Control of the root borer has been successful with either aldrin or dieldrin sprayed on the base of the plant. DDT, chlordane, toxaphene, methoxychlor, and rhothane were

not effective at 2 pounds of toxicant per acre. Heptachlor gives some control and is being tested more extensively. Dieldrin applied to the base of the mat only will give good control for about two years at the rate of 2 pounds toxicant per acre. Aldrin at the same rate will give faster control but the residual effects are not as lasting as with dieldrin. The application of dieldrin granules for red rust thrips control has given some control of the borer. Dieldrin as a fruit spray for thrips control does not control the borer since very little of the material goes into the soil.

The banana stalk borer, *Castniomera humboldti*, is a pest of bananas in Costa Rica, Panama, Colombia, and Ecuador. The insect has been known for several years in Colombia and Ecuador and within the past year has been found in the banana regions of Central America. Darlington (1929) studied the stalk borer in Colombia in 1928 but since it has not been an economic pest very little work has been done on it since that time.

The eggs are laid on the small suckers and are usually deposited behind the outer leaf sheath. Immediately after hatching, the larvae bore into the plant where they feed at random in the rhizome and gradually work their way up into the pseudostem of the mother plant. The larval period lasts from 4 to 5 months and the mature larva is nearly three inches long. The larval tunneling can cause severe damage or death to the plant and even a plant with a light infestation of stalk borer will not produce a stem of quality fruit. The pupal stage which is passed in the pseudostem, usually close to the outer surface, requires about 30 days. The adult is a large, fast-flying, diurnal moth, most frequently seen about mid-day.

At the present time, the only effective means of controlling the stalk borer is by farm sanitation: that is, banana stumps must be cut close to the ground, fallen plants quartered to hasten deterioration, drains kept free of grass and weeds, and the vegetation within the plantation chopped close to the ground. Chemical control is not recommended. Any spray that would interfere with the ant population, believed to be an important factor in controlling this insect, would probably be more detrimental than beneficial. Several insecticides, including botanicals, inorganic and synthetic organic chemicals are being screened, but results are not yet available.

Scale insects are generally of minor importance in banana production. Under the relatively dry conditions found in the Dominican Republic and in Honduras at certain times of the year, both the coconut scale and the Florida red scale increase to a point where some fruit will be discarded because of them. Oil sprays are used in the Dominican Republic but in Honduras control is effected by ladybird beetles *Pentilia castanea* Muls. and *Scymnus* sp.

In Central America, Florida red scale populations are very sensitive to rainfall and as the rains increase, red scale populations decline. The reverse is true of coconut scale which increases during the rainy season when a fungus attacks the predatory ladybird beetles.

The tortricid fruit-scarring caterpillar, *Platynota rostrana* (Walker), is usually of minor importance although at times the populations increase to a point where the fruit is not suitable for market. The damage caused by this pest is the result of the feeding of the larvae on the outer peel of the banana to produce a scabbing, usually near the tip of the finger and extending toward the base. Life history studies indicate that the larval period is from 35 to 40 days while the egg and pupal stages are usually 8 to 10 days each, or a total of 50 to 60 days for the life cycle to be completed.

Perthane at the rate of one pound toxicant per acre applied as a spray to the hanging fruit has proved an effective control measure against the fruit-scarring caterpillar. A second application 10 to 12 days after the first is necessary to obtain complete control. The larvae are subject to attack by the reduviid, *Castolus plagiaticollis* Stal., which under most conditions effectively controls this pest.

The leaf-feeding caterpillar, *Ceramidia butleri*, occurs sporadically and is generally controlled by a chalcid egg parasite, *Trichogramma minutum* Ashmead. In areas of high population, up to 86% of the eggs have been parasitized. The larvae are parasitized by an unidentified tachinid while the pupae have been parasitized by an unidentified ichneumonid wasp. Dieldrin sprays to the leaves have been more successful in reducing the number of parasites than the number of caterpillars. Egg counts made two weeks after an application



of dieldrin showed only 12% parasitized in the sprayed area as against 86% in the unsprayed area. Bordeaux is partially effective when applied to the underside of the leaf since the residue acts as a repellent to the larvae. The residue deposited on the unhatched eggs is toxic to the newly hatched larvae as the chorion of the egg is their first food.

Another leaf-feeding caterpillar, *Caligo* sp., was recently encountered in Ecuador. The mature larva of this pest attains a length of approximately four inches and is capable of eating a large amount of leaf surface. This caterpillar is apparently controlled by a fungus which attacks the pupae. Of several chrysalids collected, all were dead and exhibited symptoms of fungal parasitism.

The flower thrips, *Frankliniella parvula* Hood, is found throughout Central America. This insect oviposits in the peel of the young fruit, producing a small pimple. After the larvae hatch, they go immediately to the flowers to feed. No further damage is done to the fully formed fingers of fruit and the small blemish is fairly inconspicuous in the ripened mature fruit.

Another flower thrips, *Frankliniella musaeperda* Hood, found in the Dominican Republic, has been known to cause enough damage to the fruit to render it unmarketable. The oviposition marks turn black and increase in size as the fruit approaches maturity. Partial control has been obtained by bagging the very young fruit but this practice is expensive.

Insecticides are assuming an ever-increasing importance in solving the entomological problems of banana production. However, application of insecticides is not the only or complete answer to these problems. In areas treated with dieldrin granules for red rust thrips control, the thrips were completely eliminated but so were the more beneficial insects, such as ants. An application of dieldrin granules has been followed after about three months by an outbreak of the fruit-scarring caterpillar. Natural enemies of the caterpillar re-establish themselves in from 3 to 5 months and during this period, other sprays are needed to control this pest. In spraying the hanging stem of fruit for control of red rust thrips, care must be taken to spray only the fruit and not the lower leaves where most of the red scale and ladybird beetles are found. If the spray is carelessly applied and the leaves are covered, the predators will be destroyed and the scale will increase.

The application of dieldrin granules was associated with the present outbreak of banana stalk borer in Panama and Costa Rica. The ants and other natural enemies which generally prey on the eggs and other stages of the borer were eliminated, which allowed the caterpillar to increase to devastating proportions. The borer was present in seemingly insignificant numbers prior to the application of dieldrin but as soon as its natural enemies were reduced it became a pest of economic importance.

Another result of the dieldrin application in Costa Rica is the appearance of the small chalcid wasp *Orasema costaricensis* Wheeler & Wheeler, which oviposits in the peel of immature fingers of fruit. About a year after the application when the toxic residue in the soil had been reduced to a point where the ants could again survive, the black ant began to return and was able to re-establish itself in abundance. Newly hatched planidia of the wasp attached themselves to the ants foraging on the fruit and were carried back to the ant nest where they parasitized the developing ant larvae or pupae. Thus, the adult wasps, which apparently can lay many thousands of eggs, were numerous enough to seriously damage a large number of stems of fruit hanging in the vicinity of the ant nests. No control has been necessary to date but it is believed that a local spray of some material such as aldrin or dieldrin applied to the ant nests in the immediate vicinity of the damaged fruit would be effective as the chalcid adults are not strong flyers and their range is limited.

In the production of bananas, as in the production of any other major crop today, the application of insecticides is a necessary operation. However, the materials must be used with discrimination. Large-scale applications of insecticides, if not carefully applied, will tend to augment the entomological problems rather than reduce them. Biological and cultural control would appear to be the most satisfactory. It is known that ladybird beetles aid in controlling the scale; that the reduviid aids in controlling the fruit-scarring caterpillar; that ants aid in controlling the stalk borer; and that farm sanitation or cultural control also aids in the control of the stalk borer and the root borer. The natural agencies should be put to work as the prime factor in insect control and insecticides should be used only to supplement this factor.

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# The Sawfly Element in the Insect Fauna of Fruit Plantations in Britain

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## ABSTRACT

The mixed weed flora of young fruit plantations harbours a wide range of sawflies; as the trees mature they attract increasing numbers of their peculiar species. In young plantations *Dolerids*, *Hoplocampids* and a few nematine sawflies occur. In plum damson, and cherry plantations, and on Myrobalan, *Nematus lucidus* and several species of *Priophorus* and *Cladius* may be found. Where berry fruits are grown in young tree-fruit plantations, *Cladius* spp., *Emphytus*, and at least three species of nematines occur. *Blennocampa* may be present on strawberry and *Empria tridens* on raspberry. The Allantine *Ametastegia glabrata* F. has long been recognized as a member of the orchard fauna. Species specialized to particular orchard crops, and infesting the fruit, include several *Hoplocampids*.

The vegetation of a long-term fruit plantation in Britain usually becomes stabilized as a grass orchard in a period as short as seven or eight years in the case of dessert apples or as long as fifteen to twenty years in the case of pears, plums and cherries. There is now a tendency to shorten these periods in both groups with the result that the insect fauna of the plantation will correspondingly achieve some measure of stability in a shorter period.

As long as arable cultivations are maintained in the plantation, so long will the mixed weed flora harbour a wide range of sawflies and at the same time the fruit trees, with their increasing permanence and growth, will attract more and more the species specialised to them. In young plantations *Dolerids* and *Hoplocampids* and a few nematine sawflies may be found on the trees or in the blossom trusses.

The *Dolerids* are grass feeders in their larval stage and have come to the trees for pollen, nectar, leaf exudate, aphids or aphid honeydew. After the blossoming period they disappear from the arable orchard, moving off to grass verges, hedgesides, and grass fields. In plum damson and cherry plantations, in addition to the *Dolerids*, adults of *Nematus lucidus*, and several species of *Priophorus* and *Cladius* occur. As larvae this group feeds largely on *Prunus*, *Crataegus* and *Rosa*, so may be expected on plums and cherries as well as on damsons, where these are used as windbreaks, or on myrobalan, *Prunus spinosa* (sloe) and *Crataegus* where these constitute the surrounding hedges.

Where berry fruits are included in the early stages of young tree-fruit plantations, *Cladius* species occur, together with *Emphytus* and at least three species of Nematine sawflies. As larvae these are all leaf-eaters. Two other species that may be present are *Blennocampa* on strawberry and *Empria tridens* on raspberry.

The Allantine species, *Ametastegia glabrata* F. has long been recognised as a member of the orchard fauna and though its larvae feed exclusively on polygonaceous plants they attract the grower's attention only when, in seeking hibernating sites in autumn, they puncture the ripening fruit.

## THE HOPLOCAMPA SPECIES

A list of species specialised to particular orchard crops and infesting the fruit would include several *Hoplocampids* (see below). These latter are univoltine so that adults and larvae are only seen at blossoming time and while the young fruits are still swelling. The genus is of widespread occurrence and is richly represented in America. The hosts and flight periods of the species occurring in British orchards are as follows:

<i>Hoplocampa testudinea</i> Kl.	Apple	April—May
<i>H. brevis</i> Kl.	Pear	April 17th—28th (A. M. Massee)
<i>H. flava</i> L.	Plum	April 6th—May 6th
<i>H. chrysorrhoea</i> Kl.	<i>Prunus</i>	April 18th—May 12th
	<i>cerasus</i>	
<i>H. rutilicornis</i> Kl.	" "	April—May

*Hoplocampa testudinea* and *H. flava* occur fairly regularly in Britain and probably cause economic loss; *H. brevis* on pear has only been taken in numbers in Cambridgeshire and Kent. England has as yet no great acreage of pears (not much over 10,000 acres) but should the conditions favour the insect it might become much more widely distributed and troublesome than at present. *H. chrysorrhoea* though widely distributed in Britain has not so far been recorded as attacking commercial crops. The adults are usually taken in April and May and have been found at pear blossom and at cherry blossom. The usual source, however, is sloe, and specimens from Scotland, from Lancashire, Cheshire and the Western Counties have been examined as well as five taken on one occasion at East Malling, Kent by A. M. Masee. The species may well become more common, for R. B. Benson (1940) records the species from four localities in Hertfordshire and Buckinghamshire. It is possible that it breeds on plum and the larvae are confused with those of *H. flava*. More work on rearing is therefore desirable. My table (Miles, 1936a) for the identification of the adults is helpful in determining the species but little has been done on the specific characters of the larvae.

*Hoplocampa rutilicornis* Kl. occurs rarely in Britain. It, also, is found on sloe blossom and in the flowers of plum and damson.

The fact that at least ten species of *Hoplocampa* occur in Britain and Western Europe and over twenty species in America, and that they are associated with Rosaceae, many of which are in cultivation, suggests that some species may be expected in apple and pear orchards and in plantations where plums and cherries are grown and damsons or other species of *Prunus* used in shelter belts.

### THE CLADIINE SAWFLIES

In the Cladiini, *Priophorus varipes* Lep. occurs fairly regularly in the larval stage attacking plum and cherry foliage. There may be two generations in the summer and adults may be taken from April to August. The species is widespread in Britain and has been freely recorded by observers since Theobald's (1909) early record. May and June are still the most usual months for finding the larvae. Perkins (1929) and Benson (1940) both give host plants other than *Prunus* and related genera, and mention birch and beech. I have not reared larvae from these last two plant species, but they may well harbour segregates worthy of recognition. Dr. Herbert Ross (1951), too, recognises the difficulty with this genus and has retained a list of at least seven species.

To the fruit grower the larvae may occur as locally troublesome defoliators on *Prunus* and occasionally on *Rubus* but his usual spray programme is likely to keep them under control.

The *Cladius* complex still puzzles investigators on both sides of the Atlantic. The bristly larvae of at least two species, or forms, occur on *Rubus* and *Fragaria* and may be found in numbers feeding on the foliage of commercially grown strawberries and blackberries. Differences in habit and biology on these and related host plants suggest segregation from *C. difformis* Panz., but until more rearing work has been done the accepted relationship of *C. pectinicornis* Geoffroy with rose and strawberry and *C. difformis* Panz. with rose and blackberry may be retained.

With both species, in Britain several generations occur from April to September and maiden strawberries and runner beds are sometimes heavily attacked and the routine use of DDT or BHC gives control.

Another green and spiny larva found occasionally on cultivated strawberry but more usually on *Spiraea*, *Rubus* and *Fragaria vesca* is that variously referred to as *Monophadnus* or *Blennocampa geniculata* Hartig. This species has been observed causing serious defoliation in Somerset (England) in an area where its other host plant, *Spiraea*, was very common (Miles, 1936b). Adults and larvae occur more or less commonly in grassy verges and hedge-sides in June and July.

### THE CALIROA SPECIES

Slugworms, the larvae of *Caliroa*, occur plentifully on the leaves of pear and cherry from June to August in Britain. The larvae usually prove to be those of *C. limacina* Retz. in Europe and *C. cerasi* L. in America. The larvae are always sluglike and tolerate full exposure to sunlight. They cause extensive damage to foliage and occasionally damage the



skin of young pears. They occur on plum and damson, but more rarely. Trees in sheltered gardens sometimes suffer regularly year after year. In Britain and northern Europe the species seems to have few natural enemies and I have reared no parasites from the larvae. Two generations a year may occur in the south of England but the larvae are easily controlled with contact insecticides and heavy infestations do not often occur in well sprayed commercial orchards. Slight infestations occur in many nurseries on maidens of pear, plum and cherry, and on the rootstock material before working.

### THE NEMATINE SAWFLIES

The Nematine sawflies that infest fruit plantations are of particular interest. *Nematus lucidus* Panz. occurs all over Britain when *Prunus* is in blossom and some adults may be beaten from the trees. Later the green larvae may be found on *Prunus* and *Crataegus*. The species is univoltine so that it is only in May, June and early July that larvae are at all plentiful.

In areas where *Prunus spinosa* abounds and where *Crataegus* is used in the plantation hedges some attack on plum and damson leaves is to be expected (Miles, 1936c).

Two representatives of the genus *Micronematus* Konow occur on fruit trees in Britain, and a curious gregarious species, *M. gregarius* Marl., in America. The British species are *M. abbreviatus* Hart., which I have taken on several occasions on pear, and there are also good records by Perkins (1929) and by Benson (1935), thus indicating general distribution, and *M. monogyniae* Hart. on prunus. Both species occur generally in Britain and the larvae may be found occasionally in numbers in sheltered gardens. Both appear to be univoltine and are not likely to cause trouble under commercial conditions.

The Nematines also include several *Ribes*-infesting sawflies of which there are four important species in Britain. Three of these fall within Ross's *Ribesii* group, *Nematus ribesii* Scop. of world wide distribution wherever the host plant is grown, *N. leucotrochus* Hartig, a less known species on gooseberry, and *N. olfaciens* Benson, which infests *R. nigrum* (Benson, 1953). The fourth species is the well-known sawfly with pale green unicolorous larvae, *Pristiphora pallipes* Lep., now regarded by Ross as synonymous with *rufipes* of the same author. This insect has been known since early in the nineteenth century as a troublesome pest of gooseberries. It occurs fairly regularly in Britain; it depends largely on parthenogenesis and produces female offspring by this means. *P. pallipes*, *N. ribesii* and *N. leucotrochus* attack red currant but the new species *N. olfaciens* has so far been found only on black currant. The occurrence of this last mentioned species in localities far apart in England and Scotland suggest that it may appear in commercial plantations anywhere in Britain. It has several generations in the year and like *ribesii* produces males by parthenogenesis.

With all these leaf-eating species early recognition of an impending attack and the prompt application of insecticide will usually give good control and check an outbreak.

### THE EMPHYTINE SAWFLIES

This review is not exhaustive and I am not able to include the leaf mining sawflies (Fenusini) nor the social sawflies (*Neurotoma* spp.), but there is one other group of sawflies that merits brief mention, viz., those generally referred to as the Emphytini in the broad sense. Here we have the species *Emphytis cinctus* L., well known to American and European entomologists as a pest of roses and strawberries. It has an extensive literature and the pest inspection services of most countries know its larval habits of cocooning in the pruning snags of roses. In Britain it may have two generations in the year and its larvae are sometimes destructive to young foliage in strawberry runner beds.

In Europe larvae closely resembling those of *E. cinctus* sometimes occur on raspberry and cause extensive defoliation. These are the smooth green caterpillars of *Empria tridens* Kon. Dr. Ross retains this generic name and gives at least one species, *E. maculata* (Norton), as occurring in America on "*Fragaria* and other Rosaceae". It seems likely that larvae of *Empria* may well occur in commercial plantings of strawberry and raspberry but its presence may be overlooked.

Of special interest to apple growers throughout the Northern Hemisphere is the species *Ametastegia glabrata* Fall. This sawfly feeds on *Rumex* and *Plantago* and pupates in herbaceous or soft woody stems. It has several generations in the season. The more

these host plants appear in the orchard herbage the greater will be the risk in autumn from the mature larvae tunnelling into apples when in search of hibernating sites. The adults are plentiful from early May onwards but the leaf-green larvae are elusive and may be easily overlooked in the orchard. When orchards are put down to grass and gangmown regularly, dock, sorrel and plantain may resist the mower for a time and survive in the sward, but the docks will grow increasingly in the trunk squares, where the gangmower does not reach and where it is difficult and costly to cut by handmower, scythe or hook. These conditions result in a steadily increasing food supply for the insects and therefore the dock sawfly multiplies.

Though the number of dock plants about an orchard may not seem great it is surprising how many occur even in a moderately well-kept plantation. A winter inspection of 257 trees in a Kent orchard between 20-30 years old showed a total of 240 well-established dock plants, within a three-foot radius of the trunks. These plants had an average of five old flowering stems per plant. About 8% of these hard, dry stems contained the hibernating caterpillars of the dock sawfly and one plant with a total of eighteen stems contained six larvae. However, in a sample of thirty-five hibernating larvae, in their winter chambers in the stems, twenty-one proved to be parasitised or had already been destroyed by parasites that then occupied the sawfly chambers. This suggests that control applied in winter might easily kill more parasites than sawflies.

### CONCLUSIONS

Studies in Britain and elsewhere have shown that sawflies representative of about thirteen genera or over twenty species may occur in fruit plantations and cause some loss in yield or quality of produce. While routine control measures probably keep most sawflies in check further investigations on the distribution, biology and habits of the several species are necessary if insecticides are to be used economically and efficiently and general methods of orchard hygiene are to be sensibly employed.

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# Insects of the Coconut Palm in Surinam

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## ABSTRACT<sup>1</sup>

The results of a survey of insect species occurring on the coconut palm in Surinam are given; 20 species are arranged according to their economic importance. The author deals mainly with data concerning the life history and the chemical control of the insect pests of major importance. The following grouping is proposed:—

### I INSECT PESTS OF MAJOR IMPORTANCE

Four insect species can be recorded in order of importance: the stem-boring caterpillar *Castnia daedalus* Cram., the stem-attacking beetle *Strategus aloeus* L., the leaf-destroying caterpillar *Brassolis sophorae* L., and the scale-insect *Aspidiotus destructor* Sign.

### II INSECTS OF MINOR IMPORTANCE

The slug caterpillars *Euprosterna elaeasa* Dyar and *Sibine fusca* Stoll, the caterpillars *Caligo oedipus fruhstorferi* Stich. and *Opsiphanes quiteria* Cr., the aphid *Cerataphis variabilis* H.R.L., the stingless social bee *Trigona hyalinata* var. *branneri* Ckll., and, from single records only, the following species: *Pseudococcus nipae* Mask., *Vinsonia stellifera* Westw., *Aleurodicus cocois* Curt., *Rhynchophorus palmarum* L., and *Rhina barbirostris* F.

### III INSECTS OF NO APPARENT IMPORTANCE

The scale insects *Ischnaspis longirostris* Sign., *Chrysomphalus ficus* Ashm., and *C. dictyospermi* Morg., an *Anotia* sp. (fam. *Derbidae*), and the tree ant *Azteca chartifex* For.

## DISCUSSION

L. C. SCARAMUZZA. Has any investigation been made on the parasites of *Castnia* or *Brassolis*? I remember that in 1933, Dr. Myers published some comments on *Palpozenillia palpalis* attacking *Castinia licoides* in *Heliconia* in British Guiana.

J. B. M. VAN DINTHER. We have already found three parasites of *Brassolis sophorae*: egg-parasite *Telenomus migrocoxalis* Ashm.; caterpillar-parasite *Winthemia pinguis* Reinhard; pupal-parasite *Brachymeria incerta* Cress. Of *Castnia daedalus* one egg parasite is known: an *Ooencyrtus* sp. (fam. *Encyrtidae*).

<sup>1</sup> Full article published in *Agr. Expt. Sta. Surinam Bull.* 69, 1956.



# Some Insects of Importance on Basic Food Crops in the Mexican Tropics

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## ABSTRACT

*Early in 1955 agricultural research was initiated at two new locations in the Mexican tropics. One of these locations in the State of Sonora, at Ciudad Obregón, is in an area characterized by a dry, hot climate with little rainfall and an occasional winter frost. The other is on the gulf coast of Mexico, 30 kilometers south of the city of Veracruz. Here the climate is quite different with both hot dry, and hot wet seasons. There are heavy rains during the summer months and no frosts occur.*

*The general research programs at these stations have been directed toward the development of food crops adapted to the soils and climates of these regions. In Sonora, work has started with corn, wheat, sorghums, and horticultural crops. In Veracruz, studies with beans and rice have also been included.*

*The chief objective of the first year's work in entomology at these stations was to determine the insects that might be pests of economic importance. A series of systematic observations was made on the insects that appeared and the nature of their damage to the crops grown. Several species were present in numbers sufficient to indicate that they may be limiting factors in the production of basic food crops in the Mexican tropics if measures are not developed to control them. This paper is a summary of the observations and results to date.*

Early in 1955 agricultural research was initiated on newly established experiment stations of the Mexican Ministry of Agriculture at two locations in the Mexican tropics. The general research programs at these research centers have been directed toward the development of food crops adapted to the soils and climate of these regions. The chief objective of the first year's work in entomology at these locations was to determine what insects might be pests of economic importance on these crops. A series of systematic observations was made of the insects that were present and the nature of their injury. Following this survey, studies have been started to obtain more exact estimates of the damage caused by the more prevalent pests as well as more information on their biology and ecology, aimed toward the development of appropriate control measures. This paper is essentially a summary of the first year's observations.

Before proceeding to the discussion of the individual insect problems a brief description of the areas where the observations were made is in order.

One of these new centers of research is in the State of Sonora, near Ciudad Obregón, and is actually outside the tropical zone about 275 miles north of the Tropic of Cancer. This area has a dry hot climate with about 8 inches of rainfall annually and an occasional winter frost. The major part of this rain occurs in the months of July and August. The mean temperature is 80°F. with maximums as high as 112°F. and lows of about freezing. The altitude at this station is about 250 feet. The natural vegetation on uncleared land in the area consists of cactus, brush and some grasses.

Ciudad Obregón is in a newly developed area of rapidly expanding agriculture. The major crops grown at present, all under irrigation, are cotton during the hot summer months and spring wheat during the winter. Some corn is being grown on a commercial scale for the first time this summer and fall. In the future there will be a demand for the introduction of more crops as the area and need for diversification continue to grow. The farmers here operate large holdings, are mechanized and can afford the use of chemical insect control measures where needed. Work has started on the experiment station at Ciudad Obregón with corn, wheat, sorghums, beans and some horticultural crops.

The second new center of research at Cotaxtla, is on the gulf coast of Mexico, 20 miles south of the city of Veracruz. Here the climate is somewhat different, with both hot dry and hot wet seasons. The mean annual rainfall is slightly over 60 inches with the major part falling during July, August and September. The mean temperature is 75°F. with highs of 93-95° and lows in the high 40's. The climate is further complicated with



hurricanes during the fall and strong windstorms from the north in the winter months. The natural vegetation consists of palms, tropical grasses, and thorny scrub brush.

The farmers in this area of Mexico operate on small holdings where they have been growing corn during the summer and beans during the fall months for many years. These farmers are poor and operate their farms with oxen and hand labor. Work has started at the Cotaxtla experiment station to develop better varieties of corn, beans, sorghums, rice and horticultural crops for the area.

The following report of the insects observed on the crops at these stations has been organized by the crops involved. Corn being the chief food crop of Mexico, we shall discuss the pests on this crop first.

### CORN PESTS

The four most prevalent insects found infesting corn on both experimental stations were the stalk borer, *Diatraea* spp., the fall armyworm, *Laphygma frugiperda* (J. E. Smith), the corn earworm *Heliothis zea* (Boddie) and leafhoppers of the genus *Dalbulus*.

In corn grown during the normal growing season under natural rainfall conditions at Cotaxtla during the summer of 1955, 75 to 85 percent of the plants were infested with the stalk borer. In later plantings at the end of the wet season few borers were found. Corn this summer is again infested to levels of 85 percent. All stalk borer specimens determined to date from this station have been the neotropical corn stalk borer, *Diatraea lineolata* (Wlk). At Obregón the late summer plantings of last year had about 10 percent of the stalks damaged by the southwestern corn borer, *Diatraea grandiosella* Dyar. There were few borers present in later plantings.

The damage caused by these two species is very similar. The adults deposit their eggs at night, either singly or in groups of 2 or 3 on both sides of the leaves. The larvae feed for a time in the whorl before they tunnel into the stalk where they complete their development and pupate.

The injury to the plants is of two types. When small plants are attacked the leaves are perforated by the larvae feeding in the whorl and the growing point may be damaged or destroyed causing a dead heart or stunted plant. These plants do not produce a crop. When larger plants are attacked the chief damage results from the tunneling of the larvae within the stalk, weakening it so winds may blow the plant over before the corn has matured. In plants that remain standing the yield is probably reduced. We are, at present, trying to obtain estimates of the extent of this type of loss, to determine what control measures can be economically feasible.

Chemical control does not appear promising for this pest in Cotaxtla as it is expensive and it is also difficult to maintain a deposit on the plants during the heavy rains. The use of biological control appears more promising. Several parasites of *Diatraea lineolata* (Wlk) occur at Cotaxtla. We have found a *Trichogramma* species attacking the eggs and an *Apanteles* species destroying the larvae. We also collected a Tachinid fly, *Paratheresia* spp. and a Chalcid wasp from the pupae.

In Obregón, where corn is new in the area, our problems with the stalk borer are not great at present. However, it is highly probable the populations will increase with the expansion of corn acreage in the area.

Populations of the fall armyworm, *Laphygma frugiperda* (J. E. Smith), have varied at the two stations with from 30 to 90 percent of the plants affected by the larvae feeding the developing whorls of the plants. The first year's observations would indicate that populations are highest in the spring at Cotaxtla and during the fall in Obregón.

The adult moths of this species deposit their eggs on the leaves near the whorl of the plants and the young larvae consume each other and plant tissue, ragging the leaves as they unfold. Usually one mature larva is found in each plant.

Although this budworm damage looks severe in small plants, the resultant reductions in yield are probably small. In our work we have not been able to detect sufficient yield reductions to outweigh the cost of the 1 to 2 applications of DDT or toxaphene that have given good control of this insect.

During the summer at Cotaxtla large populations of the fall armyworm develop on the lush growth of grasses that come with the rain. This summer these larvae moved over to corn in the spike stage causing severe damage. Control measures will probably be neces-

sary to protect plants from this armyworm attack. Plants that were 8 inches to 1 foot tall when this attack occurred were severely injured before the worms were controlled with one application of DDT. Now the plants appear to have outgrown this early defoliation.

The corn earworm, *Heliothis zea* (Boddie), has not been a problem in Cotaxtla to date. However at Obregón there was severe damage last fall from a large population that developed on the corn in late August and September. Most of the damage was caused by the typical feeding of the larvae on the silks and developing ears. Some larvae were noted feeding on the foliage. This insect appears to be one of the two most important pests on corn in the Obregón area.

The second pest that has caused severe damage to corn at the Obregón station area has been leafhoppers of the genus *Dalbulus*. These are important vectors of the corn stunt virus in Mexico. On corn planted last December, populations of 150 leafhoppers per plant developed and there was a 95 percent incidence of corn stunt. This corn yielded only 200 pounds per acre. The populations of leafhoppers have decreased in later plantings but have been sufficient to cause a high incidence of corn stunt.

At Cotaxtla the leafhopper population of the same genus has been low in all plantings, 2 to 3 per plant. However in corn planted in September of last year, the incidence of corn stunt was 45 percent. The answer to this problem probably lies in the development of corns resistant to the corn stunt disease.

At both stations, in addition to the four insects mentioned above, thrips of the genus *Frankliniella* and Chrysomelid beetles of the genus *Diabrotica* have appeared on corn in the seedling stage. Also aphids have been present on the tassels of more mature corn. Little damage has resulted from these insects.

### WHEAT PESTS

A second of the basic food crops of Mexico is wheat. During the winter of 1954-55 serious damage was caused in the commercial wheat crop in the Obregón area by the English grain aphid, *Macrosiphum granarium* (Kirby). Where large numbers of these aphids appeared on the wheat heads when the grain was in the late milk or soft dough stage, the yield was appreciably reduced and in some limited areas the crop was destroyed. This last winter we were prepared to make more exact studies of the damage caused by this pest, but an infestation failed to develop at Obregón. Limited information from a study made in another location indicates that losses in yield begin to result when the population reaches 50 aphids per head on wheat in the late milk stage. No wheat has been grown at the Cotaxtla station.

### BEAN PESTS

Beans are the third major food crop in Mexico. We have made observations on the pests of beans for one growing season in Cotaxtla and are just starting the work in Obregón. In Veracruz beans normally are planted at the end of the rainy season and the crop is produced on residual moisture. Chrysomelid beetles of the genus *Diabrotica* caused severe damage to the beans grown at the Cotaxtla experiment station last fall. The adult beetles almost completely defoliated the seedlings and no crop was produced by those plants that were not treated with insecticides. These beetles also fed on the flowers of more mature plants. To date we have collected 13 distinct species of Chrysomelid beetles at Cotaxtla. The most abundant last fall and winter on the bean was the banded cucumber beetle, *Diabrotica balteata* Lec. We have obtained good control of this pest with 1 and 2 applications of DDT at 1 pound actual per acre.

A second pest of beans in Cotaxtla has been leafhoppers of the genus *Empoasca*. Injury from this insect was not severe on the early plantings last fall where applications of insecticides were made, but on a small trial conducted early this spring the plants were destroyed by leafhoppers. The injury was the typical curling and drying of the leaves.

### OTHER CROPS

On rice at Cotaxtla we have had no serious insect problems though a Pentatomid and Tettigoniid have been observed in small numbers feeding on this crop.

There have been minor populations of aphids on the heads of sorghum at both stations.

As far as horticultural crops are concerned we have had the squash vine borer and a Pyralid larva infesting the squash at Cotaxtla. There have been some thrips on the onions in Obregón and practically none in Cotaxtla. The fruit of tomatoes in Cotaxtla has been damaged by the larva of a Pyralid moth.

#### DISCUSSION

L. C. SCARAMUZZA. I wonder if the "corn stunt" of which Dr. Young spoke is the same as "corn stripe", a mosaic disease that we have in Cuba and is transmitted by *Peregrinus maidis*.

DOUGLAS BARNES. No. The most characteristic symptom of the corn stunt disease is the stunting and very faint clear stripe on the leaves. These are two types of corn stunt which have slightly different symptoms.

# Insects of Cultivated Plants in the Central Highlands of New Guinea

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## ABSTRACT

From 92 insects, found on various cultivated plants in the Eastern and Western Highlands, 72 represent new economic records for the Territory of Papua and New Guinea and 69 for the South Pacific Island Area (See the map of this area in L. J. Dumbleton: "A list of insect pest recorded in South Pacific Territories").

Ten of the 92 species can be considered major pests, all of the order Coleoptera. These are: the Melolonthid *Lepidiota vogeli* Brenske (pest of Gramineae), the Dynastid *Papuana* sp. (Pest of various vegetables), the Cyladid *Cylas formicarius* F. (Pest of Sweet Potato), the Curculionids *Apirocalus cornutus* Pasc. and *Aulacophrys fascialis* MSHL., and five new species of curculionids.

## INTRODUCTION

Before 1954 very little was known of insects associated with cultivated plants in the Eastern and Western Highland Districts of the Territory of New Guinea. After the last war some systematical collecting was carried out by N. Blood and T. Gilliard. Large numbers of Lepidoptera (mainly Rhopalocera) were collected in the late forties by W. Brandt in the Western Highlands and in 1955 very intensive collecting of various insect orders was carried out in both Eastern and Western Highlands by Dr. J. L. Gressitt (Bernice P. Bishop Museum, Honolulu.)

The Highland areas are the most densely populated areas of New Guinea, with sweet potato as a staple diet in the subsistence agriculture. In addition, during the last 6-7 years, considerable progress has been made in the extension of the European settlement with coffee (*Coffea arabica*), potato (*Solanum tuberosum*), and vegetables of the temperate climate as the main lines of activity. The culture of coffee by the indigenees has also been fostered by the Department of Agriculture, Stock and Fisheries. As a result of this more than 1000 village plantations were established in the Eastern and Western Highlands. Most coffee plantations were established in the valleys of larger rivers (Asaro, Bena, Waghi) between the altitudes of 5000 and 6000 feet above sea level.

With the growing coffee industry, with the establishment of more and more plantations and village coffee blocks, the number of economic entomological problems is rapidly growing from year to year and more and more planters show keen interest in agricultural entomology, taking notice even of the appearance of insignificant minor pests in their plantations and in their gardens.

The multiplicity of the entomological problems in the Highlands of New Guinea is connected with the zoogeographical character of this densely populated area. Besides some semi-cosmopolitan, tropo-politan, oriental and Australian elements there are many endemic insects in the fauna of the New Guinea Highlands and quite a number of species of economic importance are restricted to very small areas, to certain valleys, mountain groups, and altitudes. Most of these stenothermous and stenotopous species are extremely polyphagous. In some ways, their polyphagous nature is an advantage from the economic point of view, because the damage caused by them is not concentrated on one single plant species, as it is in the case of the monophagous species or to a moderate number of related plant species, as it is in the case of the oligophagous insects; but through their habit of feeding on 20-30 or more plant species, the injury caused by them is divided between several cultivated and many more indigenous wild plants and noxious weeds. Many Curculionids, Galerucids, Hemipterons and some Lepidopterons in the New Guinea Highlands represent typical examples of polyphagia.

The disadvantage of the polyphagous nature is the possibility of an easy spreading of these pests from native vegetable gardens and from the secondary grass-land, strongly infested with dangerous, partly introduced weeds, to the neighbouring plantations. It was many times proved during the last two years (1954-56) that much can be done in the



control of the apterous polyphagous insects with the maintenance of proper plantation hygiene in the coffee blocks. Growing of adequate temporary and permanent shade, regular cutting of weeds between the coffee bushes and the shade lines, regular pruning of the trees and cutting of the lower branches, mulching of the ground etc. will keep away many pests from the coffee blocks; or at least reduce the density of their populations in the plantation.

The above described zoogeographical character of the Central Highlands gives a formidable task to the economic entomologist in respect of chemical and biological control methods, because the nature of the various pests of every small area has to be studied separately; in many cases even near related species show a different grade of susceptibility to the same insecticide or are controlled by distinct species of parasites and predators.

Since July 1954, the writer has visited the Highlands several times, spending there altogether four months. During these visits, many insects were collected on cultivated plants, including some parasites and predators. A small part of the collected insects could be identified in the Laboratory of the Department of Agriculture at Port Moresby and at Lowlands Agricultural Experiment Station, Keravat (New Britain); but the bulk of the material was forwarded for identification to the Commonwealth Institute of Entomology, London. Some specimens were sent to American and European specialists. Most of the collected pests, parasites, and predators were identified by the specialists of the Commonwealth Institute of Entomology and the British Museum. Appreciation is expressed to Dr. W. J. Hall, the Director of the Commonwealth Institute of Entomology, to Dr. China, the Keeper of Entomology at the British Museum and to the specialist taxonomists for their helpful cooperation.

In the following chapter all insects are listed, which were found in association with cultivated plants and also their parasites and predators. As was expected, a number of the insect pests represented new species. Specimens of these were retained by specialists for description.

## ENUMERATION OF PESTS, PARASITES AND PREDATORS

### ORTHOPTERA

#### ACRIDIIDAE

*Stenocatantnos angustifrons* Walk. This species was found as a minor pest of the foliage of *Coffea arabica* in various plantations in the Eastern Highlands. It is of strongly polyphagous nature and it was observed feeding on various weeds and shrubs in the secondary grass-land on the terraces of the Asaro Valley.

#### TETTIGONIIDAE

*Phaneroptera brevis* Serv. Minor leaf pest of young coffee bushes and seedlings in the Bena Valley and in the Waghi Valley. Other species of the subfamily Phaneropterinae are recorded as pests of tea, sugarcane, tobacco and other crops at higher altitudes in Java (Kalshoven 1951, p. 123-124).

#### GRYLLIDAE

*Brachytrypes achatinus* Sauss. This species was found to be injurious to young coffee bushes at Kinjibi Plantation, in the Western Highlands. The bite marks were found on the stem from 1½" under the ground to a height of 2" above the ground. The bite marks had a rather asymmetrical form and the older ones were partly covered with callus-tissue. Some of the young trees showed a set-back in growth, as a result of the bark damage, caused by the strong mandibulae of the large brown cricket. A large number of cricket holes was found between the coffee bushes and the *Crotalaria* shade lines and the adults and nymphs of *Brachytrypes achatinus* could be found in the ground to a depth of 3½ feet. Adults of the Carabid, *Pheropsophus intermedius* Hubth. were found associated with the various nymphal stages and the adults of the cricket and it is suspected that this is one of its predators. Another species, *Brachytrypes portentosus* Licht., is mentioned by Kalshoven (1951, p. 140) to be causing similar damage to *Coffea arabica* in Indonesia. The applying of insecticides, especially stomach poisons in the form of baits, against *Brachytrypes achatinus* at Kinjibi appeared to be risky, because the inhabitants of this area, including the labourers at the plantation eat the large adults of this species. However, when the noxious insect



began to spread in the plantation, the planter gave a serious warning to the indigenees and he eventually controlled the *Achatinus* outbreak by a  $\frac{1}{2}\%$  Dieldrin spray.

*Acheta commoda* Walk. Found on two occasions causing some bark and root-damage to coffee seedlings in the Waghi Valley (Western Highlands) and at Aiyura (Eastern Highlands).

#### GRYLLOTALPIDAE

*Gryllotalpa africana* Pal. Found to be causing some root-damage to coffee seedlings in the nurseries in various plantations of the Asaro Valley.

### HEMIPTERA (HETEROPTERA)

#### PLATYASPIDAE

*Coptosoma* sp. Found in small numbers on *Crotalaria anagyroides* and *Coffea arabica* at Goroka (Eastern Highlands, 5200 feet).

#### PENTATOMIDAE

*Nezara viridula* L. This semi-cosmopolitan pest was found on tomato, beans, green peas, *Crotalaria anagyroides* shade-trees and on *Coffea arabica* in various localities, but it did not appear to cause serious damage in the Highlands.

*Plautia affinis* Dall. On *Coffea arabica* in the Asaro Valley.

*Melanacanthus* sp. On *Coffea arabica* in the Eastern Highlands.

*Oncocoris* sp. On *Coffea arabica* in the Waghi Valley (Western Highlands) and in the Kainantu Subdistrict (Eastern Highlands).

*Philia femorata* Walk. On *Euphorbia pulcherrima* in the Asaro Valley.

*Platynopus melacanthus* Boisd. This species was found preying on various Lepidopterons, injurious to cultivated plants, e.g. *Hydroclada kenricki* B. Bak. (on *Coffea arabica* and *Lagerstræmia indica*), on *Heliothis armigera* Hbn., *Orgyia postica* Walk. and a green Geometrid larva (gen. & spec. indet.), defoliating young coffee tree.

*Canthaconidea* sp. This species was also observed preying upon the larva of the Limacodid, *Hydroclada kenricki* B. Bak.

*Aspidomus quadrimaculatus* Sign. Found several times in coffee plantations on *Coffea arabica* and *Crotalaria anagyroides*. It is believed that *Aspidomus quadrimaculatus* is a predatory species. A related species was found preying upon *Hyposidra thalaca*, a Geometrid pest of *Hibiscus cannabina* in the coastal area of Papua and New Britain.

#### COREIDAE

*Leptoglossus australis* F. This species was found to cause very serious damage to passion fruit in the Asaro Valley in 1954. It is commonly found on various Legumes and recently it was found causing serious damage to citrus fruit (Mandarins) in the Morobe District. *Leptoglossus australis* F. is a polyphagous, eurythermous species, found in almost every district of the Territory of Papua and New Guinea.

*Riptortus* spp. Two species were found feeding on the young leaves and cherries of *Coffea arabica* and on the pods of *Crotalaria anagyroides* in the Eastern Highlands.

#### COLOBATHRISTIDAE

*Phenacantha* sp. On *Camelia sinensis* at Highlands Agricultural Experiment Station Aiyura (Eastern Highlands, 5700 feet above sea level).

#### REDUVIIDAE

*Amyota glaucolimbata* Tryon. In a coffee plantation (Goroka) preying upon the larva of *Orgyia postica* Walk.

*Scipinia* sp. Found on a young coffee tree at Goroka (Eastern Highlands) preying upon the polyphagous Galerucid *Solephyma papuana* Jac.

#### LYGAEIDAE

*Nysius* sp. Found to be causing serious injury to *Helianthus annuus* in the Asaro Valley (Eastern Highlands).

## MIRIDAE (CAPSIDAE)

*Pachypeltis* sp. A major pest of *Cinchona succirubra* and *Cinchona leggeriana* and a minor pest of *Camelia sinensis*. Relatively small populations are sufficient to defoliate large areas. The completely defoliated *Cinchona* trees lose their tender branches and many young trees die. Fortunately, the species is very susceptible to DDT spray. The spraying of the trees with 2% DDT has to be repeated every 6–8 weeks.

*Lasiomiria* sp. On *Camelia sinensis* at Aiyura (Eastern Highlands).

## HOMOPTERA

The spreading of various Homopterons from the rainforest and the secondary grass land of the river valleys into the plantations was observed several times. Although some species had an extremely high population density and they literally covered the leaves and branches of some cultivated plants, they have never appeared to cause any serious damage.

## FLATIDAE

*Colgar* sp. Found in large populations on *Coffea arabica* at Kainantu (Eastern Highlands).

## JASSIDAE

*Tettigella* sp. On *Camelia sinensis* at Highlands Agricultural Experiment Station, Aiyura, 5700 feet above sea level.

*Bothrogonia* sp. A species, with yellow and black striped bright coloured wings. It was found in large numbers on *Coffea arabica* and *Camelia sinensis* at Kainantu (Eastern Highlands) and Kornfarm (Western Highlands). This species is very frequent in the moss forest area. It was found as high as 9500 feet above sea level.

*Bothrogonia* sp. This red and blue coloured species was found on *Coffea arabica* in the Kainantu Subdistrict and in the Asaro Valley, both Eastern Highlands, between the altitudes of 5200 to 6000 feet. In some plantations the coffee leaves and branches were covered with the adults of this Jassid, but no serious injury was found.

*Selenocephalus* sp. A species with light brown coloured wings. Large populations were found in one of the coffee blocks at Highlands Agricultural Experiment Station, Aiyura, at an altitude of 5700 feet above sea level.

*Selenocephalus* sp. This olive green coloured species was very abundant in all coffee blocks at Aiyura (5400 to 6000 feet). It was also found on *Crotalaria anagyroides* and *Vigna sinensis*.

## DERBIDAE

*Zoraida* sp. Found in small numbers on *Coffea arabica* and *Albizzia* sp. at Goroka (Eastern Highlands).

## APHIDIDAE

*Aphis maidis* Fitch. Observed in small numbers on the agricultural farm of the Public Health Department at Kainantu. It is most likely controlled by Hymenopterous parasites. No predatory Coccinellid larvae could be located in association with this Aphid.

*Aphis* (?) *tavaresi* Del Guercio. This species was found several times in low population densities on *Citrus* in various localities in the Eastern and Western Highlands. At the citrus orchard of the Medical Aidpost at Nondugl, the larvae of a *Callineda* sp. (Coccinellidae) and some Chrysopid larvae were observed preying on the larvae and adults of this black Aphid.

*Aphis* sp. A green species appeared recently (In June and July 1956) in larger populations on the *Crotalaria anagyroides* shade trees in some coffee plantations of the Goroka Subdistrict. Larvae of a uniformly dark yellow coloured Coccinellid, *Callineda* sp. near *testudinaria* Muls. (Det. R. D. Pope) were found preying on this Aphid.

*Toxoptera aurantii* Boisd. Found on the foliage of *Camelia sinensis* at Highlands Agricultural Experiment Station, Aiyura. The larvae of an unidentifiable Coccinellid were found as predators. This cosmopolitan Aphid was also recorded by G. S. Dun (1954) on *Theobroma cacao* in the coastal area of New Britain.

## COCCIDAE

*Coccus viridis* (Green) appears in small populations in nearly every coffee plantation in the Eastern and Western Highlands, but it seems to be very well controlled by some predatory Coccinellidae. And very likely also by Hymenopterous parasites. The larvae of the following Coccinellids were observed preying upon *Coccus viridis* R., *Pulvinaria psidii* Mark, and *Saissetia coffeae* (Walk.): *Menochilus sexmaculatus* F., *Callineda* sp. (Near *testudinaria* Muls.) and two species of *Orcus*, which according to Mr. Pope are likely to represent new species. *Coccus viridis* was also found on *Citrus* at Goroka, where it appeared to be kept under the level of economic injury by one of the above mentioned species of *Orcus*.

*Pulvinaria psidii* Mark. This species was found in many plantations in the Eastern and Western Highlands on *Coffea arabica*, but it has never appeared in real plague form. It appears that it is mainly controlled by the above mentioned species of *Callineda*. A local outbreak of both green scales, *Coccus viridis* and *Pulvinaria psidii* at Lunapieve Plantation (Upper Asaro Valley) was halted after the liberation of several thousand specimens of this *Callineda* sp., collected in the Bena Valley, at a distance of about 40 miles from the plantation.

*Saissetia coffeae* (Walk.) is also well controlled in the Highlands coffee plantations by the above mentioned *Callineda* sp. and by two species of *Orcus*.

*Saissetia* sp. Odd scales of this species were found in an orchard at Goroka (Eastern Highlands). The scales attacked citrus and mulberry trees, but the larvae of *Menochilus sexpunctatus* F. and of *Orcus* sp. seemed to keep the populations under the level of economic injury.

*Saissetia nigra* (Nietn.) This species appears in large populations on virus disease infested *Crotalaria anagyroides* shade trees in various coffee plantations of the Eastern Highlands. *Callineda* sp. was observed several times preying upon this scale, but this species does not seem to be able to reduce the populations below the level of economic injury.

*Lepidosaphes* (?) *beckii* (Newman). Found in two small Citrus Orchards at Goroka (Eastern Highlands) and Banz (Western Highlands). No parasites or predators were observed.

*Aonidiella citrina* (Coqt). Found in low densities on citrus at Banz and at Aiyura.

## PSEUDOCOCCIDAE

*Planococcus citri* (Risso). This species, first described from Southern France, has now almost a circumtropical area of distribution (see Ferris 1950, p. 165). The writer found *P. citri* on citrus at Highlands Agricultural Experiment Station Aiyura (6000 feet) and recently on *Coffea arabica*, *Leucaena glauca*, *Albizia stipulata* and *Crotalaria anagyroides* at Wau (Morobe Highlands, 3400 feet).

*Pseudococcus adonidum* (Linnaeus) [= *P. longispinus* (Targini-Torretti) (Ferris, 1950 p. 174)]. Found on *Mangifera indica* at Highlands Agricultural Experiment Station, Aiyura. The larvae of a Scydmin Coccinellid (similar to those of *Cryptolaemus mountrouzieri*) were observed preying upon the adults but no adult Coccinellids could be located.

*Icerya* sp. One citrus tree was found to be infested with this large yellow species in the orchard of the Roman Catholic Mission at Banz (Western Highlands). Adults of a *Coelophora* sp. were seen in large numbers on the same tree but no larvae could be located. The larvae of another unidentifiable Coccinellid were observed preying upon the *Icerya*. It is likely that this species is related to an *Icerya* sp. recorded by Kalshofen (1951, p. 353) from the Highlands of West Java and Sumatra.

## LEPIDOPTERA

## TORTRICIDAE

*Homona coffearia* Nietn. This species was found in small numbers in most coffee plantations in both Eastern and Western Highlands. A serious outbreak in a young coffee block at Koffena Plantation (Upper Asaro Valley) was controlled with 0.3% Dieldrin. The whole coffee block was thoroughly sprayed with the insecticide, which had a very good killing effect. *Homona coffearia* Nietn. appears to be well controlled in the New Guinea Highlands by Ichneumonid parasites. Two species (*Theronia simillima* Turn. and

*Hemipimpla clotho* Morl.) were reared from pupae by R. S. Carne and the writer. Both represent new parasite-host records. The above mentioned serious outbreak appeared during an extreme dry spell, which could have caused an upset in the host and parasite sequence.

#### LIMACODIDAE

*Hydroclada kenricki* B. Bak. This species was found defoliating *Coffea arabica* and *Lagerstroemia indica* at Goroka (Eastern Highlands). The bright coloured larvae were mainly feeding during the night and were resting on the lower surface of the leaves during the day. Two Asopin Pentatomids were observed preying upon the caterpillars of *Hydroclada kenricki*. (See under "Hemiptera").

*Pygmaeomorpha modesta* B. Bk. One coffee tree on the edge of Tremearne Plantation (Western Highlands) was strongly defoliated by the larvae of this species and of another Limacodid, which could not be identified.

#### SATURNIIDAE

*Capaxa janetta* White. The large apple green larvae of this giant silkworm were found partially defoliating *Eucalyptus deglupta* planted as ornamental tree in various gardens at Goroka (Eastern Highlands). It is parasitized by the Tachinid *Cuphocera varia sumatrensis* Tns.

#### ARCTIIDAE

*Cretonodes gangis* L. A few larvae were found by the writer on maize at Kainantu (Eastern Highlands) in October 1954. The larva is more often found in the Highlands on wild sugarcane and other Gramineae in the secondary grass land.

*Uthetesia pulchella* L. A very common species, which was found to be causing some damage to *Crotalaria* shade trees (*C. anagyroides*) in the coffee plantations, in both, Eastern and Western Highlands.

*Argina cribraria* Cl. This tiger moth is also well known as a pest of *Crotalaria* spp., but it seems to be more frequent in the coastal areas. The writer found it on *Crotalaria anagyroides* at Goroka in the Eastern Highlands and at Banz and Kinjibi in the Western Highlands.

#### AGROTIDAE

*Agrotis ipsilon* Hufn. was found to be causing some damage to garden lawns, pastures and airstrips and also to coffee seedlings in both Eastern and Western Highlands. At Lunapieve Plantation (Upper Asaro Valley) fowls were used to clear the coffee nursery from *A. ipsilon* larvae during a larger outbreak and this method proved to be successful. It can be only applied when the seedlings have reached the height of 3½ to 4 feet. During a serious outbreak at Kornfarm (Western Highlands) *Agrotis* larvae have entirely wiped out large patches of grass. Two shovels full of soil contained 3–4 larvae. Pesproof (DDT) was applied successfully against the cutworms. Hundreds of sluggish, poisoned larvae appeared on the surface of the ground shortly after the application of the insecticide. A peacock had eaten a large number of the poisoned larvae and died a few hours later. Young Casuarina trees, planted as shade trees or wind breaks in coffee plantations, are often attacked by the larvae of *Agrotis ipsilon*. An *Ichneumon* sp. was found as parasite.

*Prodenia litura* F. This widely distributed eurythermous species was found causing some injury to cabbage at Koffena Plantation in the Upper Asaro Valley and to potatoes at Lahamenigu Plantation, near Goroka (Eastern Highlands).

*Leucania unipuncta* L. Found on maize at Kainantu (Eastern Highlands.)

*Heliothis armigera* Hbn. This widely distributed species was found causing serious damage to corn cobs (*Zea mais*) at Kainantu and in a garden at Goroka. It was also found feeding on the pods of peas and beans and on the cherries of *Coffea arabica* in both the Eastern and Western Highlands.

#### LYMANTRIIDAE

*Orgyia postica* Walk. This species appears to cause some leaf damage to *Coffea arabica* in the Eastern Highlands, but it has never been found in real plague form. The Pentatomid,



*Platynopus melacanthus* Boisd., and the Reduviid, *Amyota glaucolimbata* Tryon, were observed preying upon larvae of *Orgyia postica*.

#### SPHINGIDAE

*Herse convolvuli* L. This species is known to cause very serious damage to sweet potato in the coastal areas of New Guinea. Although many adult moths were collected by the writer above garden flowers and round electric lamps in the Highlands, it could not be found causing economic injury in this area. A few larvae were found in a village garden near Goroka feeding on *Ipomoea batatas*. It is very likely that *Herse convolvuli* is well controlled in the Highlands by one of the many large Ichneumonids, found in this area.

#### SATYRIDAE

*Melanitis leda* F. A few larvae of this widely distributed species were found on *Zea mais* near the airstrip at Goroka.

#### NYMPHALIDAE

*Eriboea pyrrhus jupiter* Butl. The larvae of this butterfly were found defoliating *Albizzia* shade trees at Bayer River (Western Highlands). A female was observed laying its eggs on a Jacaranda tree in an ornamental garden at Goroka.

#### LYCAENIDAE

*Polyommatus baeticus* L. The larvae feed in the pods of *Crotalaria anagyroides* shade trees in the coffee plantations. Although large numbers of adults were observed in most plantations, the damage caused by the larvae was unremarkable.

#### PAPILIONIDAE

*Papilio aegeus ormenus* Guerin. This is one of the commonest butterflies of the cultivated areas in the Eastern and Western Highlands. The larvae appear to feed mainly on *Citrus* and some young trees are badly defoliated. *Echtmorpha insidiator* Smith (Ichneumonidae) and *Brachymeria* sp. (Chalcididae) were found as parasites. Their association with *Papilio aegeus* represents a new record of parasitism.

### COLEOPTERA

#### TELEPHORIDAE

*Chauliognathus waroensis* Wittmer. This is one of the commonest beetles in *Coffea arabica* plantations in the Eastern Highlands of New Guinea. It is found in large numbers on both coffee bushes and *Crotalaria* shade trees. It is a beneficial insect, which was found preying upon various polyphagous Lepidoptera larvae (*Heliothis armigera*, *Orgyia postica*, Geometrid larvae).

#### COCCINELLIDAE

*Orcus* sp. (nova?) (Det. R. D. Pope). Predator of soft scales.

*Orcus* sp. (nova?) (Det. R. D. Pope). Same as the previous species.

*Callineda* sp. near *testudinaria* Muls. (Det. R. D. Pope). Effective predator of Aphids and some soft scales.

*Menochilus sexpunctatus* F. Predator of soft scales.

Some unidentified Coccinellids were found preying upon *Icerya* sp., *Pseudococcus adonidum* and *Toxoptera aurantii*.

#### LAGRIIDAE

*Ceragria* sp. This species was observed by J. Barrie and the writer feeding on the leaves of *Crotalaria anagyroides* and of young coffee bushes. It was also found by A. Schindler on *Camelia sinensis* at Highlands Agricultural Experiment Station Aiyura.

#### TENEBRIONIDAE

*Gonocephalum biroi* Kasz. The larvae of this species were found causing some injury to the roots of coffee seedlings in the nursery at Lahamenigu Plantation (Asaro Valley, Eastern Highlands). Similar damage was found on coffee seedlings in other nurseries, where the adult beetles were very abundant at ground level.



*Pseudolyprops serrimargo* Gebien. Found in large numbers under young coffee bushes at Kinjibi Plantation (Western Highlands). It is very likely that the larva causes similar injury to coffee roots as it was found in the case of the previous species.

*Pseudolyprops szent-wanyi* Kaszabi. Found in coffee plantations around the roots of young coffee bushes.

#### LAMIIDAE

*Pterolophia mediochracea* Brug. Found in various coffee plantations in the Eastern Highlands. The larva is a suspected stemborer of young coffee trees.

#### GALERUCIDAE

*Monolepta* sp. On the foliage of *Camelia sinensis* at Highlands Agricultural Experiment Station, Aiyura.

*Aulacophora pallidifasciata* Jacq. On the foliage of *Coffea arabica* at Kinjibi and Aiyura and on *Ipomoea batatas* in the Asaro Valley.

*Aulacophora papuana* Jac. Found on *Coffea arabica* and *Ipomoea batatas* in the Eastern Highlands. This seems to be an eurythermous and strongly polyphagous species. It was found by the writer also on *Theobroma cacao*, *Phaseolus* and *Cucurbitaceae* in the coastal area of New Guinea.

*Solephyma papuana* Jac. Another polyphagous species. Found on *Camelia sinensis* at Aiyura, on *Coffea arabica* at Goroka, on pumpkin and sweet potato in the Upper Asaro Valley.

*Morocasia* spec. nova (Det. G. E. Bryant). On *Camelia sinensis* at Highlands Agricultural Experiment Station, Aiyura.

*Ceratia* sp. On pumpkin at Kainantu (Eastern Highlands).

#### EUMOLPIDAE

*Rhyparida coriacea* Jac. This eurythermous species causes slight defoliation of *Coffea arabica* and *Citrus* in various parts of the Eastern Highlands. The writer found it on various ornamental trees and shrubs (*Euphorbia pulcherrima*, *Lagerstroemia* spp., *Acalypha*) in the gardens of the Goroka town area and G. S. Dun (1954) recorded it as a leaf pest of *Theobroma cacao* in the coastal area (also see Dumbleton 1954).

#### DYNASTIDAE

*Xylotrupes* sp. The adults appear from time to time in very large populations round *Poinciana regia* trees in the ornamental gardens at Goroka and they cause considerable damage by scratching the bark of the lateral branches.

*Papuana* sp. The adult beetle represents a major pest of cabbage, potato, strawberry and turnip in the Eastern Highlands. The beetles chew the tubers of the potato, feed on the roots of the turnip and on the strawberry fruits. They crawl up on the stem of the cabbage, miss two to three leaves and then they bore tunnels in the middle of the cabbage head. More than 10 specimens can be found in one single cavity. The larvae feed on decaying matter in the ground and Mr. G. Gilmore, plantation owner at Koffena (Eastern Highlands) succeeded in controlling the larvae with a 0.3% Dieldrin spray. Its suspected parasite is *Scolia punctatissima*, a species always found in large populations in areas which are infested with the grubs of this *Papuana* sp.

#### MELOLONTHIDAE

*Lepidiota vogeli* Brenske. The larva of this species is a dangerous pest of pastures, garden lawns, golf courses and airstrips. The grub feeds on the roots of the grass. As a result of this the grass dies in large patches and the surface of the ground becomes boggy, obstructing the landing of the airplanes on the airstrips. The Commonwealth Department of Civil Aviation was considering the closing of the airstrip at Goroka after the serious outbreak of *Lepidiota vogeli* in 1953. This could have adversely affected the Highland coffee industry and the whole economic situation in the Highlands of New Guinea, as there is no road connection between the Central Highlands and the coastal area and the airstrip at Goroka is the largest and most important in the whole Highland area. Everywhere in the Eastern Highlands *Lepidiota vogeli* Brenske is associated with the Scoliid wasps, *Campsomeris formosus* Guer. and *Campsomeris tasmaniensis* Sauss., which are believed to be

its ectoparasites. During a series of cage experiments at Goroka it was observed that the females of these two Scoliids readily attack and paralyze the larvae of *Lepidiota vogeli*. On account of this and of some field observations it was recommended to the Department of Civil Aviation to attract the adult wasps by the planting of adequate garden flowers round the airstrips. As a result of this and possibly of some unknown microclimatic conditions the density of Scoliid populations have remarkably increased during the last two years in the Asaro Valley and apparently as a result of this the condition of the air field and of garden lawns and pastures in the Goroka area has considerably improved.

Three insecticide tests were carried out against *Lepidiota vogeli* Brenske during the years 1954 and 1955, one on the side of the Goroka airstrip and two on the training grounds of the Police Depot at North Goroka. Aldrin, Dieldrin, Chlordane and DDT were applied in these experiments. The two experiments on the police training grounds had a very encouraging result during the first three-four weeks. Aldrin and Dieldrin brought the grub populations to a low density in nearly all experimental plots, where these two insecticides were applied, but towards the end of the fourth week the populations of the control plots began to decrease and finally they also came down to the same level. The application of insecticides against *Lepidiota vogeli* in the Eastern Highlands is extremely risky as the adult beetles are strongly favoured by the indigenous inhabitants. The swarming of the adult beetles lasts three to four days only and they usually swarm at dusk or exceptionally, during the day in very dark cloudy weather. During the swarming time, hundreds of Highlanders appear on the pastures, garden lawns and on the airstrips. They collect the beetles in bottles, cook them in open fire and eat them on the same day. One of the officers of the Department of Agriculture has tasted the roasted beetles and he found them quite palatable.

#### CURCULIONIDAE

*Apirocalus cornutus* Pasc. This is an extremely polyphagous species, which was found in many parts of the coastal area of New Guinea, but in the Highlands it seems to be restricted to the relatively small area of the Kainantu Subdistrict, where it occurs between the altitudes of 5400 to 6000 feet. It causes quite considerable injury to *Coffea arabica* by the chewing of the top shoots of young tender bushes. *Apirocalus cornutus* Pasc. was found feeding on the following plants in the Kainantu Subdistrict: *Coffea arabica*, *Camelia sinensis*, *Cassia alata*, *Cinchona succirubra*, *Cinchona leggeriana*, *Grevillea robusta*, *Crotalaria anagyroides*, *Euphorbia pulcherrima*, *Cucurbita pepo*, *Zea mais*, *Carica papaya*, *Mangifera indica*, *Paspalum conjugatum* and *Panicum palmarum*. The writer has found it on many more host plants (*Theobroma cacao*, *Musa sapientum*, *Manihot utillissima*, *Coffea robusta*, *Hevea brasiliensis* etc.) in various parts of the Madang, Morobe Districts and the Central District of Papua. It is very likely that this species has invaded the Highlands through the Markham valley.

*Aulacophrys fascialis* Mshl. The genus and the species were recently described by Sir Guy A. K. Marshall. It is one of the typical polyphagous and stenothermous elements of the Highlands fauna, mentioned in the first chapter of this paper. *Aulacophrys fascialis* MSHL. seems to be restricted to the Kainantu Subdistrict. It causes quite considerable shot hole damage to *Coffea arabica*, *Camelia sinensis* and to the seedlings of *Cinchona succirubra* at Aiyura. Besides this it was found feeding on *Citrus*, *Mangifera indica*, *Panicum palmarum*, *Verbena* sp., *Crotalaria anagyroides* and on many forest trees.

*Oribius destructor* Marshall. This species replaces *Aulacophrys fascialis*\* and *Apirocalus cornutus* in the Asaro Valley and in a part of the Western Highlands. It is extremely polyphagous and it is considered to be one of the major pests of *Coffea arabica* in the Asaro Valley. Fortunately, it can be controlled with DDT, BHC or Dieldrin. The following hostplants were noted: *Coffea arabica*, *Citrus*, *Albizia stipulata*, *Albizia* sp., *Jacaranda*, *Crotalaria anagyroides*, *Euphorbia pulcherrima*, *Lagerstroemia indica*, *Lagerstroemia* sp., *Passiflora* sp., *Morus* sp., *Acalypha*, *Ipomoea batatas*, *Brassica oleracea*, and on many noxious weeds. The larvae are found in the ground round the roots of various Gramineae and Compositae. The adults were also observed chewing the surface of coffee cherries.

\*Described by Sir Guy A. K. Marshall as *Oribius Destructor*. Bull. Ent. Res. London, Vol. 48, p. 3-4.

*Oribius hostis* Marshall. This is another polyphagous and stenotopus species which causes quite considerable damage to *Coffea arabica* in a very restricted area of the Western Highlands.

*Celeuthetini* sp. nova (Det. E. C. Zimmerman). Similar in habits to the previous species. It is restricted to the Banz-Nondugl area of the Waghi Valley.

*Cryptorrhynchini* sp. A species, identified by E. C. Zimmerman as genus near *Acales*, appeared as a major pest in some plantations of the Goroka Subdistrict. Another apparently related species causes similar damage to *Coffea arabica* in the Kainantu Subdistrict. The larvae of both species attack the bark of the coffee trees on the main stem (exceptionally also the lateral branches) causing typical "ring-marks" or "girdler marks". As a result of this the younger trees show a serious set-back in growth, the leaves are drooping and wilting and some branches die off. It very often happens that the stem thickens above the girdler mark and if the cambium is strongly injured, the wind breaks the stem in half under the thickened area. Most of the broken trees can be saved with adequate pruning. The counting of girdler marks on 320 trees in the worst infested area of a plantation at Goroka had the following results:

1. From 320 trees 250 had girdler marks.
2. The total number of girdler marks was 592.
3. A 7 feet tall coffee bush had 12 girdler marks on its main stem.

Dieldrin and DDT were tested in an insecticide trial carried out in 1955 at Bena Plantation (Goroka Subdistrict). As a result of this test, it was found that the *Cryptorrhynchini* Girdler can be controlled by spraying the whole infested area (the coffee bushes, the shade trees and the ground), if the larvae are very young, in the very beginning of their life cycle. Dieldrin showed a very good killing and a long lasting residual effect. 1% DDT had also a fairly good killing effect, but its residual effect did not last as long as the residual effect of the 0.5% Dieldrin. The application of Dieldrin and DDT in such a high concentration is rather uneconomical. Further insecticide experiments have to be carried out in the future with some more insecticides and with various concentrations.

#### CYLADIDAE

*Cylas formicarius* F. A serious outbreak of the sweet potato weevil appeared during the extreme dry season of 1954 in the Goroka and Chimbu Subdistricts of the Eastern Highlands. In some village areas the reduction of yield was as high as 60% as it was estimated by agricultural officers R. S. Carne, R. Cottel and J. Sharpe. Very interesting observations were made by Mr. R. P. Reilly on his farm situated near Goroka on a lower terrace of the Asaro Valley. A 60 acre-block of *Ipomoea batatas* was seriously attacked by *Cylas formicarius* and it was noticed that the whole crop of the white skinned variety was practically lost, while the interplanted red skinned variety showed a 100% resistancy. The sweet potato crop in a block of 100 acres was literally wiped out by *Cylas formicarius* at Wau, in the Morobe Highlands during the extreme dry season of 1954. It was several times observed in New Guinea that the most serious outbreaks of *Cylas formicarius* have appeared in relatively large blocks, earned by European settlers, where *Ipomoea batatas* was repeatedly planted throughout several growing periods. Very rarely were found large scale infestations in the smaller garden blocks of the villagers, who have the habit of abandoning their gardens after a few years of cultivation and opening up new areas in the rain-forest.

#### DIPTERA

##### AGROMYZIDAE

*Agromyza* (?) *coffae* Knb. Odd leaves, attacked by this species, were found in most plantations in the Central Highlands. It is likely that this Agromyzid is controlled by parasites or has some other subsidiary host plants in the Highland areas.

##### TACHINIDAE

*Cuphocera varia sumatrensis* Tns. Found as an endoparasite of the Saturniid, *Capaxa janetta* White, at Goroka (Eastern Highlands).

##### ASILIDAE

*Maira* sp. Observed preying on the adult of *Heliothis armigera* at Bena Plantation. (Goroka Subdistrict).

## HYMENOPTERA

## ICHNEUMONIDAE

*Ichneumon* sp. Parasite of *Agrotis ipsilon* Hufn.

*Echtmorpha insidiator* Smith. Found as a parasite of the Citrus Butterfly, *Papilio aegeus ormenus* Guerin, at Goroka, Eastern Highlands.

*Hemipimpla clotho* Morl. Parasite of *Homona coffearia* Nietn.

*Theronia simillima* Turn. Parasite of *Homona coffearia*. All Ichneumonids were determined by Mr. G. J. Kerrich.

## CHALCIDIDAE

*Brachymeria* sp. A relatively large species of the genus, reared from pupae of *Papilio aegeus ormenus* Guerin at Goroka, Eastern Highlands.

## FORMICIDAE

Species of the following genera were found damaging the lawns of a golf course at Goroka: *Odontomachus* sp., *Iridomyrmex* sp., *Paratrachina* sp., *Paratrachina* (Subgenus *Nylanderis*) sp., *Aligomyrmex* sp., *Euprenolepis* sp., *Polyrachis* (Subgenus: *Cyrtomyrmex* sp.) The identification was made by Dr. E. O. Wilson (Harvard University, Boston) during his visit to New Guinea in 1955.

## VESPIDAE

*Rhopalidia* sp. Nests of this species were often found on the foliage of *Coffea arabica* in various plantations in the Eastern Highlands.

*Polistes comis* Ch. Their nests are even more numerous in coffee plantations than those of the previous species.

## SCOLIIDAE

*Campsomeris tasmaniensis* Sauss. Suspected ectoparasite of the larvae of the grass-pest *Lepidiota vogeli* Brenske (Melolonthidae).

*Campsomeris formosus* Guer. Suspected ectoparasite of *Lepidiota vogeli* Brenske.

*Scolia punctatissima*. Suspected ectoparasite of the larvae of the noxious vegetable pest *Papuana* sp. (Dynastidae).

## MEGACHILIDAE

*Megachile lachesis* sp. This species was found damaging the foliage of *Coffea arabica* and of various ornamental garden trees and shrubs (*Eucalyptus deglupta*, *Lagerstroemia*, *Rosa*) in the Eastern Highlands.

*Megachile* sp. Large numbers of ground nests of this species were found on garden lawns and on the tennis court at Kinjibi Plantation (Western Highlands).

## APIDAE

*Nomia pulchribalteata* Cam. This small bee has built thousands of ground nests in the garden at Kinjibi Plantation in 1955, entirely wiping out the grass in the garden and on the lawn tennis court.

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# Sannhemp and its Insect Fauna

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## ABSTRACT

*Sannhemp*, *Crotalaria juncea*, is an important green manuring crop in the State. The important caterpillars which defoliate and bore into pods are, *Utetheisa pulchella*, *Argina cribraria* and *Argina syringa*. All these occur in association with each other. Caterpillars of *Laspeyresia tricenra* and *Cymotricha tetraschema* form galls on the shoots and stems of sannhemp, thereby reducing and affecting the growth. Other minor pests are *Ragmus importunitas*, *Longitarsus belgaumensis*, *Amyna octo* and *Polyommatus boeticus*. Extensive studies on the galls of sannhemp revealed that heavy damage is done to the crop. Gall formation is as high as 75%. Most of the galls occur on the growing points and obstruct the linear, healthy growth of the plant. The gall formation usually leads to side branching.

*Sannhemp*, *Crotalaria juncea*, is one of the important fibre yielding crops in India and is grown in the State of Hyderabad over an area of about 90,000 acres. In Hyderabad, however, sannhemp is grown largely as a green manure crop, mostly in the project areas where perennial irrigation facilities are available. It is also a very good fodder crop, and is fed both green and dried like hay or is grazed in the field.

Sannhemp is attacked by quite a number of pests. Among the hairy caterpillars, the most important are recorded below.

*Utetheisa pulchella* (Arctidae).—This is the most important pest of the sannhemp crop in the state. The hairy caterpillar is not only a defoliater, it bores into the seed capsules as well. The attack usually starts when the crop is one month old. Severe infestations by the pest may result in complete defoliation, leaving only bare stems. At later stages caterpillars bore into the pod and feed on the seeds. While feeding on the pod the caterpillar never completely enters it, only thrusting its head into the capsule.

*Argina cribraria* and *Argina syringa* (Hypsidae) are the two other hairy caterpillars which sometimes cause damage to the sannhemp crop. These pests are usually found in association with *Utetheisa pulchella*. The feeding habits of all these hairy caterpillars are similar.

*Laspeyresia tricenra* (Olethreutidae).—This is a small caterpillar and the adult a small greyish-brown moth. It bores into the stems and branches of sannhemp near the nodes causing swellings which assume gall-like shapes. In certain seasons this is a very serious pest causing considerable losses, especially to sannhemp grown for fibre. The pupa is found inside the gall.

*Cymotricha tetraschema* (Gelechiidae).—The caterpillars bore into the shoots and stems of sannhemp causing galls. This results in a reduction of growth and considerable losses are caused when the attack is severe. Sannhemp is a new host for this pest. Pupation takes place in the gall.

The sannhemp capsid, *Ragmus importunitas* (Capsidae), is a serious pest of sannhemp and causes appreciable damage. It attacks in swarms, sucking up the plant juice and causing a chlorotic appearance. When infestations are severe the entire field appears whitish instead of green. Attacks usually start when the crop is about a month old.

Minor but sometimes important pests on sannhemp are the flea beetle, *Longitarsus belgaumensis* (Chrysomelidae), and the green semilooper, *Amyna octo* (Noctuidae). Flea beetles usually attack the crop when it is very young and continue till it is about a month old. In the early stages they scrape chlorophyll, making the leaves look like tissue paper, and also bite small holes in the leaves. The green semilooper is rarely a pest. It usually occurs in association with other caterpillars.

Other minor pests on the crop are caterpillars of the blue butterfly, *Polyommatus boeticus* (Lycaenidae), and *Etiella zinckenella* (Pyralidae), which bore into the pods. These occur along with other pod-boring caterpillars.

As *Laspeyresia tricenra* and *Cymotricha tetraschema* cause galls in sannhemp, field observations were made on the extent of gall formation, the position of galls on the plant and

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the parasitism of gall forming insects. A summary of the data obtained appears in Table I. Records were also taken regarding the damage caused to the pods by pod borers.

TABLE I—Summary Data on Galls of Sannhemp. All Figures Represent Percentages.

No. series	Galled plant	Galled branches per plant	Position of galls on plant				Parasitism in gall
			Growing point	Nodes	Lower stem	Upper stem	
1.	79	17	70	8	3	19	48
2.	69	20	71	6	4	19	80
3.	60	17	69	6	5	20	62
4.	86	8	73	7	3	17	52
5.	85	14	83	9	1	7	63
Average	75.8	15.2	73.2	7.2	3.2	16.4	61

Both *Laspeyresia* and *Cymotricha* cause galls on sannhemp. During 1952–1953, heavy attack was noticed on the crop and the percentage of galled plants was as high as 75. Records were taken by counting the healthy and galled plants in randomly selected 3 x 3 subplots in 5 different series; the total number of plants counted was 640. The number of galls per plant varied between 0–10. They vary in shape from globular to oblong. They are hard when found on the stem and soft when found at growing points. Small holes for the escape of adults are found on the surfaces of the galls. Old galls cracked and there was profuse branching from the site of the gall. Heavily galled plants put forth numerous short shoots, their growth being reduced. In no case did death of the plant result from gall formation. Galls could be found on all aerial portions excepting the leaves and flowers.

During observations it was noticed that galls occurred mostly in the terminal portions of the plants. Oblong swellings on the stem near the soil were broken or cracked in many cases. Shoots and the growing points had fresh galls from which adults were reared. Galls on the main stem either on the lower or upper side had become hard. About 73% of galls were formed at about the growing point and about 16% on the upper portion of the stem. Nearly 7% were found on the nodes and 3% on the lower portion of the stem. The details are clearly seen in the table.

A glance at the table reveals that galls are formed mostly at the apex, which shows that insects are more active during the latter part of the season. Infestation is lightest when the crop is young, later building up with the growth of the plant.

Although 75% of the plants were found with galls, the proportion of galled branches in a plant was much less. Random counting of the galled shoots in a plant was done in 5 series involving 800 individual plants. Only 15% of the branches were found with galls when the number of branches in a plant varied between 7 and 8.

When galls are opened small white silken cocoons may be found. Usually only one cocoon is found per gall, but in two cases two cocoons were seen in a gall. Hundreds of galls were examined and it was found that 61% had silken cocoons of *Apanteles taragamae* (Braconidae). This high percentage of parasitism was noticed at a time when the infestation was also very high. A few specimens of *Atherigona* sp. (Chamaemyiidae) were also reared from the galls.

The caterpillars of *Utetheisa*, *Argina*, *Polyommatus*, and *Etiella* bore into the pods and feed on the seed. Seed production becomes very important in areas where sannhemp is grown for green manure purposes. Extensive records show that, on an average, 18% damage is caused to the capsules, making them unfit for seed.

#### ACKNOWLEDGEMENT

Grateful acknowledgement is made to Dr. M. Q. Khan and Dr. F. C. W. Muesebeck for facilities and for arranging the identification of parasites.

# Some Important Agricultural Pest Problems of the West Midland Province in England

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## ABSTRACT

*An attempt is made to sketch a part of the contemporary Advisory scene. The use of insecticidal seed dressings against the chief pests, wireworm (*Agriotes* spp), carrot fly (*Psila rosae* F.), flea beetles (*Phyllotreta* spp), for which they are effective is reviewed. Methods of dealing with pests not amenable to such treatment, namely, the wheat bulb fly (*Leptohylemia coarctata* Fall), leatherjackets (*Tipula* spp), the frit fly (*Oscinella frit* L.), including forecasts and surveys are briefly referred to. Finally, the increasing importance of the oat or cereal root eelworm (*Heterodera major* O Schmidt) in some parts of the province and a line of work being followed are described.*

The West Midland Province of England consists of the six counties Cheshire, Shropshire, Herefordshire, Staffordshire, Warwickshire, and Worcestershire. The province is bounded on the northeast by the foothills of the Pennines and on the west by the Welsh highlands. Between lies the Cheshire and North Shropshire plain—the gap of the Dee and Severn Valleys. To the south-west lie the Cotswolds but the east is open to the central plain—a popular invasion route from the earliest times—and still used by cabbage white butterflies whose annual migrations across England from the Continent of Europe keep going a pest of occasional severity which otherwise might well be controlled by natural parasites.

The outlying hills of the province are mainly concerned with sheep and cattle rearing. Cheshire is a dairying county and so largely is Staffordshire. The Shropshire plain already mentioned contains some of the best farmed land in England and is largely mixed arable with the accent on dairying. All the main cereals are grown and there are large areas of potatoes and sugarbeet. The Triassic sandstone soils provide fine carrot land. To the south of the province general mixed farming is practised with a fair amount of fruit of which blackcurrants are of increasing importance as a source of a vitamin C syrup. Hops are another specialised crop here and the Vale of Evesham watered by Shakespeare's Avon is a famed market-garden area. Market gardening is indeed an important industry since many millions of England's population are concentrated (or congested) in three large industrial areas, the Manchester region to the north, the Potteries and Birmingham and the Black Country in the centre and south-east. Almost all the geological formations from the Precambrian to the Jurassic are represented but the most important agricultural soils are mainly derived from the Triassic sandstones and marls, the old Red Sandstone with smaller areas of the Lias and the Carboniferous. Overlying these formations, mainly in the northern half of the province is an incomplete cover of Glacial Drift, which in places is quite extensive.

It is obvious therefore that with such a variety of crops and soils, entomological problems are likely to be of a very varied character also.

Undoubtedly the two innovations that have most altered the impact of the major pest problems in general agriculture are the development of the insecticide-containing seed dressing and the low-volume sprayer. The former has put in the hands of the general farmer a cheap and convenient form of protection against several pests; the latter has made it possible for him to use many of the excellent new insecticides that have lately become available. Before the second world war there was little for the adviser to recommend beyond poison baits for such pests as cutworms, leatherjackets, and slugs. Even when an attack of a particular pest was controllable with an insecticide then in use there was no satisfactory apparatus at hand for applying it. The war enabled the firm of Pest Control to develop large-scale agricultural spraying but it was mainly for the specialised crop, e.g. sugar beet and Brussels sprouts, and (later) the specialised spray, e.g. the systemic Schradan. The introduction of the porcelain nozzle by Plant Protection gave the ordinary farmer his chance and also made him, to a point, independent of the sometimes delayed attention of the contractor. Though it is mainly used for weed sprays, it can well be used for insecticides if special care in cleaning

is exercised. It may almost be said in this regard that the wheel has turned full circle, for at the moment it is possible for a farmer to buy a sprayer at 'cost' price providing he also undertakes to buy quantities of spray material to use in it. With spray material standing in the barn there is the temptation to use it whether or no, and it has already happened that large areas of sugar beet have been unnecessarily sprayed against mangold fly (*Pegomya betae*). This point needs watching. Recently a systemic (*Metasystox*) for which no protective clothing is obligatory has become available to the general farmer, thus further extending possibilities.

### SEED DRESSINGS

The combined gamma/BHC—mercurial seed dressing has been in use for some years; in a Province that before the war was largely permanent grass and therefore an area of high wireworm population, the impact of that pest has been considerably reduced. The recent development here has been the introduction of a double-strength dressing designed to cheapen and standardise the treatment so that all cereals can be routine dressed; this makes it much easier for the seedsman without greatly adding to cost. The small amount used<sup>1</sup> (1 oz. per bushel) meant adjustments to the dressing machines to ensure even distribution: also moisture content and condition of the grain are involved—factors perhaps connected with the comparatively new combine harvesting process. However, the new treatment appeared to be quite satisfactory though occasional germination troubles were met. An extension of 'seed' treatment to the potato tuber has not yet been found practical and indeed BHC would be unsuitable because of 'tainting'. Here aldrin has found application either as a general seed-bed dressing in dust or spray form or combined with fertiliser.

But perhaps the most striking application of the seed dressing principle is against the longstanding and difficult problem of the carrot fly (*Psila rosae*) and of flea beetles (chiefly *Phyllotreta* spp). Gamma BHC is mainly used though recently dieldrin dressings have appeared. A fungicide such as TMTDS is usually added. Work on the carrot fly was begun in the West Midlands in 1946; flea beetle dressings were developed mainly from work done in Yorkshire and Wales. Though work on the carrot fly is still going on, it may be said that at present the commercial seed dressings now on the market can give adequate protection from the first generation attack. Against the second generation a dieldrin spray, which needs careful timing, is used. With flea beetle the case is much the same; seed dressings give relief from the early attacks and a spray can be used later if necessary.

Against the wheat bulb fly (*Leptohylemyia coarctata*)—which gives some trouble in the West Midlands on the heavy Lias soils after bare August fallows, though very much worse in the drier east where egg surveys are made, seed dressings<sup>2</sup> are less useful and early October sowing is still the main protection. It is interesting to note that in recent years this pest has given trouble also on the lighter soils after potatoes and broccoli and similar crops.

Against leatherjackets (*Tipula* spp.)—another grassland pest—present day seed dressings are useless and the old (Paris green) and newer (DDT and BHC) baiting methods are still the cheapest control. Low-volume spraying is, however, sometimes used. This insect is of interest in that it is one that can be fairly easily controlled, and to some extent predicted, and this matter is discussed in the next paragraph.

### SURVEYS, DAMAGE ASSESSMENT, AND FORECASTING

Population estimations and assessment of pest damage have increasingly occupied the attention of English Advisory Entomologists in recent years. This is not the place to refer at any length to this work, but merely to mention one aspect of it as it concerns two pests in the West Midlands in relation to forecasting attacks. It is possible by a fairly simple technique to get an estimate each year of the leatherjacket population. Figures for the West Midlands in 100 thousands per acre are shown in Fig. 1. It will be seen that 1952 was an epidemic year (an 'all time high' as far as the writer's experience goes) and this we were able to predict. We tried by several methods—through District Officers, newspapers and the B.B.C.—to issue warnings of the impending epidemic but with what effect it is not known. One small voice amid the babel of tongues competing for the attention of the farmer in these

<sup>1</sup> In Canada it is believed even lower quantities have been successfully used.

<sup>2</sup> Since writing this a new and stronger seed dressing, Dieldrin C, has been marketed which, it is claimed gives better results; combine drilling of aldrin has also given good results. It is thought that eventually a combined fertiliser-aldrin treatment may emerge. Aldrin-containing fertilisers for general purposes have been on the market for sometime.



days easily passes unnoticed. We do not know how many crops were saved—we certainly saw many that were not.

**LEATHER JACKET POPULATIONS**  
**WEST MIDLAND PROVINCE**  
**ANNUAL SPRING SURVEYS**

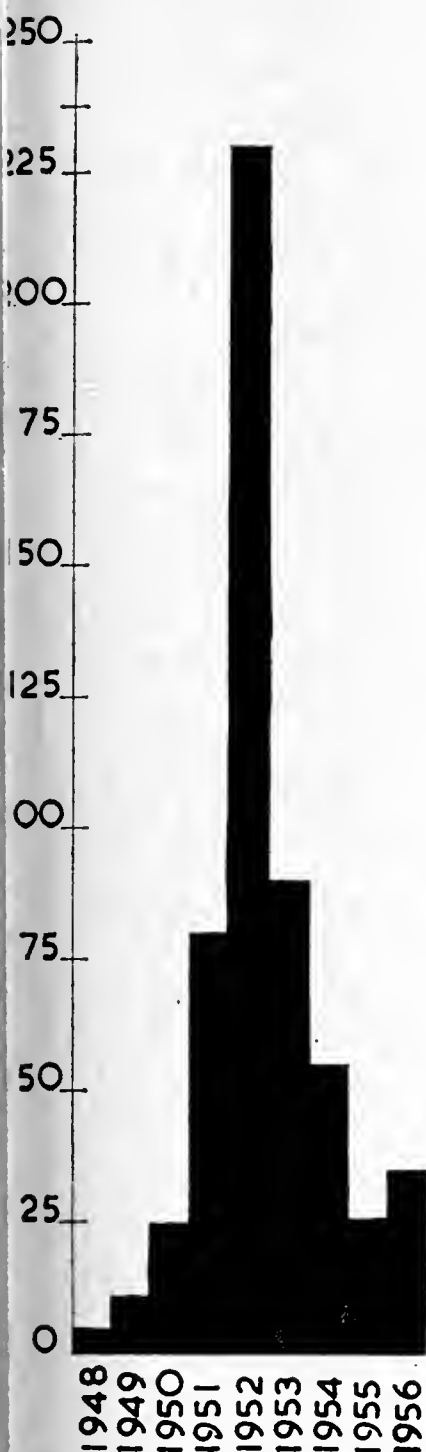


FIG. 1

**CEREAL ROOT EELWORM**  
**IN THE WEST MIDLAND PROVINCE**  
**CUMULATIVE - NEW CASES EACH SEASON IN BLACK**

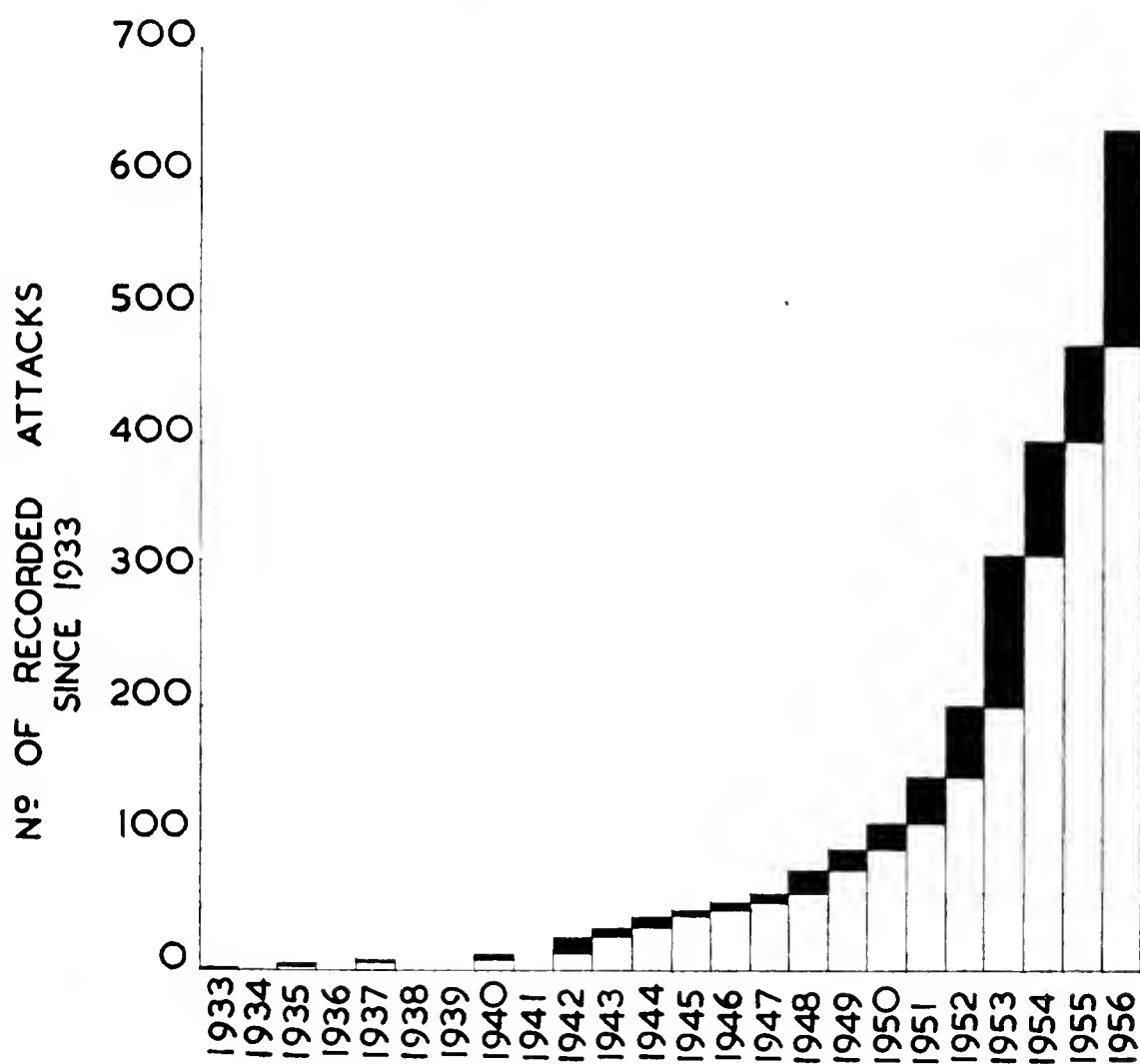


FIG. 2

Another pest on which much survey work has been done is the frit fly, *Oscinella frit*. This is mainly a pest of oats, which it attacks in the spring killing the young tillers, and again a few weeks later, in the ear, where the grains are hollowed out causing light-weight corn. We have had samples showing up to 50% of such grain attack. Following this there is a third attack on autumn cereals—again a tiller attack—but this one need not concern us here except in so far as it allows a forecast to be made of intensity for the following year.

Over the past four years small-scale national trials have been running to determine, among other things, the relation between the tiller attack and the subsequent ear attack, i.e. a comparison of protection from tiller attack, ear attack alone, and both together.

Parallel with these, large-scale trials using the half field as a unit have been carried out in the West Midlands as a direct result of requests for immediate aid from south Herefordshire where difficulty in growing satisfactory crops had been experienced for some time. To get the matter as near an economic basis as possible two tiller sprays only were tried—in any case, ear spraying is not at present practical. The result after three years, two of them during a period of low-intensity attack, seems to indicate that the increased yield will more than repay the expenditure. In the third year, 1956, attacks have been so intensive that considerable increases in yield of sprayed crops has resulted. The future work would appear to be an attempt at prediction of attack followed by the spraying of at least the later sown crops.<sup>3</sup>

<sup>3</sup> At the time of writing there is some evidence that extra strength seed dressings may also be of use here; if so, this would be a big advance.



The final pest which it is desired to refer to is not an insect at all but one of the cyst-forming eelworms, the oat or cereal root eelworm (*Heterodera major*).

It is probably the common experience that as insecticides and developments in their application become more efficient, attacks by insects alarm us less, and attacks by eelworms of various sorts take up more and more of our time. Probably over 50% of our time in the West Midlands is spent on eelworm work where we run a routine cyst count service to evaluate infestations of the different cyst-forming species, and so help the farmer in his choice of crop. Investigational and Plant Health work also involve much cyst counting.

The cereal root eelworm is a species to which we have given particular attention in the West Midlands. Our first case was recorded in 1933, since when more and more cases have turned up each year until in 1956 over 150 cases came to our notice. In 1953, 1954, and 1955 numbers were less but not greatly so (Fig. 2). These cases are failures or part failures, and there is no doubt that as District Officers and farmers have become more aware of the problem and its symptoms, fewer cases are missed, this partly accounting for the steep rise. But there is also no doubt that increased cereal cropping and possibly changes in general husbandry, have tended to build up cyst numbers in the soil. With the potato root eelworm (*H. rostochiensis*), with as far as general agriculture is concerned its single host plant, rotation is an effective weapon. With the cereal root eelworm, which will live on all the Gramineae to varying degrees, and quite successfully on wheat and barley, the word 'rotation' loses much of its meaning.

We have found that no oat is resistant enough to be worth growing on infected land, that barley is least affected, and that there may be varietal differences among the barleys that might be reflected in yields. If this should prove to be the case such varieties might also support fewer cysts and reduce build-up. Some spring wheats, notably Atle, appear to be fairly susceptible but winter wheats, e.g. Hybrid 46, are not greatly affected. Winter oats are less affected also but appreciable yield reduction may occur. Rye is hardly affected at all; nor does it appreciably raise the cyst count, but there is little demand for that crop. Most grasses also appear to drop the cyst count level.

The immediate practical outlook here is the use of propaganda to stress the importance of good husbandry and such rotation as can be practised to reduce build-up of cyst populations and the regulation of oat growing on affected land. If less susceptible varieties of barley can be found (perhaps also of wheat) that not only yield higher but also reduce cyst build-up, so much the better, and it is along these lines that we are working. Meantime the cereal root eelworm is the subject of fundamental work at both Rothamsted and Cambridge and one worker in London is investigating the possibility of control by predaceous fungi. Possibly one day a resistant oat will be bred though this is likely to be a distant prospect.

# The Turnip Maggot and Other Species of *Hylemyia* (Diptera: Muscidae) of Economic Importance in Japan

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At present, the root maggots of economic importance in Japan are represented by the turnip maggot, the onion maggot and the seed-corn maggot. Besides these three root maggots, we have another species, the smaller turnip maggot, *Hylemyia pilipyga* (Villeneuve), attacking Cruciferous crops in the northern part of Japan. Several papers in regard to taxonomic determination of these species have been published by myself since 1939. As these papers are written in Japanese, however, I wish to present here an outline of the occurrences of *Hylemyia* species attacking agricultural crops in Japan.

The turnip maggot, *Hylemyia floralis* (Fallen), is the most serious pest of the Japanese radish, *Raphanus sativus* L. var. *acanthiformis* Makino; the Chinese cabbage, *Brassica pekinensis* Rupr., is also attacked severely on occasion, but injury to the other Cruciferous crops such as turnip and cabbage is of less importance. The Japanese radish, we call it "Daikon" in Japanese, is an important vegetable to the Japanese and it is pickled for use as an indispensable winter side-dish, especially in the snowy northern parts of Japan. The root of the most common variety of "Daikon" is about a half-meter in length and some 10 centimeters in diameter, gradually tapering to a point.

The distribution of the turnip maggot is now limited to Japan's most northern Island of Hokkaido, where the life-history of the maggot and the injury caused are very similar to those found in Canada. The period of appearance of the adult fly in Hokkaido seems to be delayed about one month compared with that of Canada, the adult flies emerging from the puparia from early August to mid-September in central and southern Hokkaido. It is of interest that in the north-eastern part the adults appear about one week earlier than in other areas of Hokkaido.

Adult flies in general, and especially the females recently emerged from the puparia, have a habit of gathering around flowers of wild plants growing near "Daikon" fields, sucking the nectar from these flowers before they begin to lay their eggs. It seems that the females will not mature until they consume a sufficient amount of nectar or other substances from these wild flowers and from flowering buckwheat crops. Concerning this, it is noteworthy that the preoviposition period of this fly is comparatively long, being about seven to 10 days and that, adults fed on honey markedly prolong this period compared with flies fed only molasses or water, it being difficult to mature the latter flies artificially.

Damage in Hokkaido is so severe that it is difficult to grow "Daikon" without applying insecticides. The most effective spray used until about 1950 in controlling the maggot was a 0.5 per cent water solution of mercuric chloride. However, since then BHC, DDT and other new agricultural chemicals have been found effective, and mercuric chloride is being replaced by these.

We have not found wasps parasitising the turnip maggot in Japan, but following the request of the Dominion Parasite Laboratory, Belleville, we sent about 850 puparia of this fly to Canada in 1952. We tried also to introduce an effective parasitic wasp, *Trybliographa rapae* (Westwood), from Canada in 1953, but we failed to establish the parasite in Hokkaido; the main cause of failure was the difference in the emergence of the hosts and the parasites. I suppose that it might be possible to establish the parasite in an area where not only the turnip maggot but other kinds of root maggots, such as the cabbage maggot, occur together throughout the crop planting seasons.

Fortunately, the cabbage maggot, *Hylemyia brassicae* (Bouché), has not yet been discovered in Japan, although in the north-eastern part of Hokkaido a closely related maggot, the smaller turnip maggot, is known to cause the same type of injury to early varieties of "Daikon". The life-history of the smaller turnip maggot in Hokkaido has not been closely studied, but it has three generations a year causing severe damage to "Daikon" in early summer. It also causes severe damage in autumn when it is found in association with the

turnip maggot. The pattern of infestation between these two species on Cruciferous crops seems to be similar to that between the turnip maggot and the cabbage maggot in Europe and Canada.

The history of occurrence of the turnip maggot in Hokkaido is rather old; Dr. S. Matsumura recorded this insect as *Phorbia brassicae* or *Anthomyia brassicae* in 1897, 1898 and 1900. In 1915, he described this fly as a new species under the name of *Anthomyia flavopicta*, and this scientific name was used up to 1939, when the fly was identified for the first time in Japan as the turnip maggot.

The onion maggot, *Hylemyia antiqua* (Meigen), on the other hand, is found in scattered locations in almost all Provinces except the central area of Honshu, the main island of Japan, frequently causing serious damage in Hokkaido, Tohoku and Chugoku Provinces. The occurrence of the onion maggot was restricted to Hokkaido up to 1949, but outbreaks have since been constantly recorded from all the main islands of Honshu, Kyushu and Shikoku; it is now becoming a serious problem throughout most of Japan.

The onion maggot always over-winters in the soil in the pupal stage although the life-history, especially the number of generations in a year, shows remarkable differences between the northern and the southern provinces of Japan. In Hokkaido two generations a year are most common, although three generations can also be found. The first appearance of the adults begins about mid-May and lasts until the end of September; two peaks of emergence occur around mid-June and at the end of August, the two generations being almost continuous. In Oita Prefecture, the southernmost district in which this insect presently occurs in Japan, there are three distinct generations in a year. The adults initially appear at the beginning of March and two generations occur by the end of July; the adults of the third generation appear from mid-September to the end of October. The peaks of emergence are in mid-June and in the beginning of October, there being a complete midsummer rest in the soil.

To control the onion maggot we have, in Japan, widely adopted the treatment of applying 3% BHC dust to the base of the plants, at least three applications beginning in the early period of egg-laying. It is noteworthy that early planting of an early variety of onion shows effectiveness in preventing severe maggot damage in some areas of Japan. I consider that further studies in a particular area on the life-history of the insect, together with the cultural method of planting a specific variety of onions, seem to be necessary if an effective way to control the onion maggot is to be found.

The seed-corn maggot, *Hylemyia platura* (Meigen) [= *H. cilicrura* (Rondani)], is also widely distributed throughout Japan and seeds and seedlings of various kinds of beans, cucumber, watermelon, onion, wheat, barley, upland rice, etc. are severely infested on occasion. In northern districts of Japan, the seed-corn maggot passes the winter apparently in the pupal stage, but in the southern districts, it is reported that small numbers of adults appear and lay their eggs even in January. In general, there are two or three generations a year throughout Japan, but we can find no adults or damage caused by the larvae in any province in the midsummer season. Areas in Japan where the seed-corn maggot has only one generation a year have not yet been reported.

Regarding control measures, in addition to many cultural methods, the soil application of 3% gamma BHC dust or the dipping of seeds into a 0.0003% gamma BHC emulsion before sowing, has been widely used in Japan. Seed coating treatments with various chemicals seem to be effective; since 1955 they have been tested at the Hokkaido Agricultural Experiment Station but have not yet been extended to farmers in general.

In 1939, I published a paper on taxonomic determinations of both adults and larvae of the above mentioned flies. Since then, I have keenly felt the need for studies on the immature stages of *Hylemyia* of agricultural importance in conjunction with the studies of the adults, for the injurious stage of these flies is almost always the larva.

In conclusion, I consider that taxonomists engaged in studies concerned with agricultural entomology should contribute primarily to this field, and should regard their other studies of perhaps more personal interest, of secondary importance.

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<sup>1</sup> All these are in Japanese.





# Chemical Control of Arthropod Pests of Pastures in South East Australia

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## ABSTRACT

The relations between annual rainfall, pasture plant species and topdressing with superphosphate govern the susceptibility of pastures to insect attack. DDT, lindane, dieldrin, parathion and malathion mixed with the superphosphate fertilizer topdressing, or applied as low volume sprays, give satisfactory control of all the pests mentioned with the exception of snails.

Victorian native pastures in the 17 to 35 inch annual rainfall belt consist of perennial Wallaby grasses, *Danthonia* spp., Kangaroo grass, *Themeda triandra*, a few annual grass species of genera *Festuca* and *Bromus*, and succulent herbs of the family Geraniaceae. Sward density has been improved by ringbarking the eucalypt trees which compete for water and nutrients. Further improvement has followed the introduction of annual and perennial clovers and the practice of topdressing with superphosphate. Lime and minor elements are occasionally essential.

As rainfall diminishes, the proportion of cork-screw grasses (*Stipa* spp.) increases, and these predominate in the 10 inch annual rainfall zones. Here, pasture improvement consists of introducing exotic grasses and legumes. Where rainfall is adequate or irrigation is available, perennial white clover, *T. repens*, perennial rye grass, and cocksfoot are the favoured pasture species.

Intermediate zones of 24 inch rainfall carry perennial rye grass and annual clovers, whilst in the 14 to 22 inch rainfall belt, subterranean clover, *Trifolium subterraneum*, and annual rye grass, *Lolium subulatum*, are very extensively used. The use of superphosphate and legumes has raised the general level of soil and herbage nutrition.

Because of the extensive practice of annual topdressing of pastures, insecticides have been economically combined with superphosphates. When fertilizer has been applied, low volume sprayers are used quite effectively, although at a greater cost per acre.

*Halotydeus destructor* Tuck., the redlegged earth mite, is essentially a seedling pest and damages germinating clover, causing most severe damage in dry autumns and winters when growth is slow. Heavy rainfall is damaging to the mite population. New plantings on well prepared soil are attacked from the boundaries only, so that a narrow barrier trail of creosote prevents migration from roadsides and infested pastures to the young crop.

DDT at 4 oz., or lindane at 2 oz. per acre as a dust or mixed with superphosphate have proved highly effective, but should be withheld until after oversummering eggs have hatched and applied before the developing mites have reached the egg-laying stage. Sprays are also effective.

*Sminthurus viridis* (L.), the lucerne flea, is a collembolid which arrived in Australia last century, possibly in white clover seed. It often seriously damages lucerne, *M. sativa*, and subterranean clover, and attacks white clover. The pest is believed to be spread by eggs adhering to soil amongst seed. Seed fumigation has retarded, but not prevented, its spread throughout Victoria. A predatory mite, *Biscurus lapidus*, is believed to exercise a controlling effect.

Lindane and DDT do not give control of this species. Lime sulphur is effective; however, it is more expensive than parathion and malathion, which are both very effective at the phenomenally low dosage of one half ounce of active ingredient emulsified in 10-20 gal. of water per acre. A treatment three weeks after hatching, which occurs with the first general late autumn rains, is aimed to kill larvae and adults before egg-laying commences. Because the eggs are not affected by any of the above insecticides, treatment of later hatchings may be necessary.

*Oncopera fasciculata* Walk. is one of several native Hepialids which feed on native grasses as well as introduced grasses and clovers. Long growth is favoured by the moth for

egg-laying, which occurs in late September. The grey-black larvae feed gregariously, cutting mature growth at ground level in January. Later instars burrow extensively, forming individual burrows 4 to 9 inches deep. Very dry summers cause heavy mortality of young caterpillars. Heavy winter rainfall stirs larvae from their burrows, exposing them to the attack of insectivorous birds and the entomogenous fungus *Cordyceps gunnii*.

Light rainfall from hatching to mid winter favours the caterpillars. They destroy the seedlings, and by frequent denuding of young shoots, destroy grass crowns, so that pasture may fail completely in areas from 1 sq. chain to 100 acres.

Feeding ceases in August when the caterpillars, then 2 inches long, form dirty grey prepupae, and later, golden brown pupae. Mottled grey-brown adult moths shortly emerge.

Lindane and crude BHC have not given control of grass caterpillars. Experiments at Hamilton have shown that 12 oz. DDT per acre distributed with superphosphate gives sufficient larval mortality to protect pastures during the critical autumn period. Application should be made in February or March to prevent destruction of grass crowns and clover seedlings. Also, older larvae are less susceptible to the insecticide.

*Aphodius howitti* Hope, a scarabaeid, is another native insect which thrives on introduced plants, especially subterranean clover and ryegrass on good humus-rich soil. Subterranean clover pastures 2 to 5 years old are most susceptible. The black beetles emerge in January and seek short, heavily-grazed pastures for egg-laying.

The larvae hatch in 10 to 20 days. In feeding, the grubs burrow vigorously. After each moderate rain they bring soil to the surface, smothering much growth in the process. They feed on the roots, but to a greater extent, on the aerial parts of both grasses and clovers. Subterranean clover pastures in the 10 to 25 inch annual rainfall belts are the most susceptible.

Both DDT and lindane have proved very effective against the larvae. Lindane is recommended at 4 oz. per acre, mixed with superphosphate. It is more quickly effective against the advanced instars than DDT, and appears to be more effective than an equivalent dose of gamma isomer BHC applied in the crude form. DDT is recommended at 16 oz. per acre mixed with superphosphate, but has shown much promise at 8 oz. per acre. Natural enemies of *A. howitti* include several species of flower wasps, family Thynnidae, several predatory beetles of the family Carabidae, and the fungus *Cordyceps aphodii*.

Other pests causing damage to Victorian pastures are as follows: cutworms; the field cricket, *Acheta commodus* Walk.; the hairy Anthelid caterpillars; sod web worms, chiefly of the families Crambidae and Pyralidae; the coastal snail, *Cochicella acuta* (*Helicella barbara*); and the southern grasshopper, *Phaulacridium vittatum*. *A. commodus* is controlled by 6 oz. dieldrin per acre, in super, *P. vittatum* by sprays of 2 oz. dieldrin or 3½ oz. lindane per acre.

# The Study of Resistance to Aphids in Crop Plants<sup>1</sup>

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## ABSTRACT

There have been more known cases of breeding of crop plants resistant to aphids than to any other group of plant-feeding insects, possibly because aphids are among the most frequently observed insects on plants. Sources of resistance to aphids have involved both varieties and individual plants within a variety. Differences in preference have been observed, but rarely studied. The most commonly observed difference has been in the effect on insect fecundity and life history (antibiosis), or the effect of tolerance to the insect in plants. The methods of study in screening large numbers of plant varieties must be fitted to the behaviour of the insect and the type of reaction to be studied. The importance of food quality is shown by the fecundity on different parts of the same plant, and is probably involved in differences between plants. Preference as a repellent by taste may be important in the corn leaf aphid. Insect biotypes have been found in the corn leaf aphid on sorghums, in the pea aphid, and in the greenbug. The level and type of resistance to the several species of aphids studied differs considerably. Even the small differences appear to be worth incorporating into commercial varieties. Progress has been made on the practical use of resistance to aphids.

Plant resistance to insects is concerned with any inherited characteristic of the plant that limits the insect population or reduces the damage done.

Workers at the Kansas Agricultural Experiment Station have studied insect resistance in crop plants for thirty years. During this period 9 varieties resistant to one or more insects have been approved and distributed for use by Kansas farmers (Table I). To be approved,

TABLE I—Insect Resistant Crop Varieties Approved for Use of Farmers by Kansas Agricultural Experiment Station.

Date Approved	Crop	Varietal Name	Insect resisted	Remarks
1928	Sorghum	Atlas	Chinch bug*	Leading Sorgo in acreage in the U.S.
1932	Wheat	Kawvale	Hessian fly**	Replaced by Pawnee and removed from approved list, 1947.
1937	Alfalfa	Ladak	Pea aphid ‡	Moderate resistance
1943	Wheat	Pawnee	Hessian fly**	Developed jointly with Nebraska Agr. Expt. Sta. Leading variety in acreage, Kansas 1946. Leading variety in acreage in U.S., beginning 1948.
1943	Corn	Ks 2234	Grasshopper***	Hybrid (K55 x K41) x (K64 x K65)
1950	Corn	Ks 1859	Corn Leaf† aphid	Hybrid (N6 x WF9) x (K148 x K150); also carries moderate resistance to corn earworm††
1950	Sorghum	Dwarf Kafir 44-14	Chinch bug*	Developed for chinch bug resistance by Oklahoma Agr. Expt. Sta.
1951	Wheat	Ponca	Hessian fly**	Developed and released jointly with Oklahoma Agr. Expt. Sta.
1952	Barley	Dicktoo	Greenbug††	Developed at North Dakota Agr. Expt. Sta. for outstanding winter hardiness

\*Blissus leucopterus (Say)

\*\*Phytophaga destructor (Say)

\*\*\*Melanoplus spp.

†Rhopalosiphum maid is (Fitch)

††Toxoptera graminum (Rond.)

‡Macrosiphum pisi (Harris)

††Heliothis zea (Boddie)

<sup>1</sup>Contribution No. 673, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan.

each variety must be equal to other commonly grown varieties in most important characteristics, and superior in at least one. This research has been a joint project of the departments of Entomology and Agronomy of Kansas State College, and the United States Department of Agriculture.<sup>2</sup> Some of the studies have been made by graduate students under my direction and have been used for Masters' theses and Doctors' dissertations.<sup>3</sup>

Outstanding success has been achieved in breeding for insect resistance, especially for resistance to the hessian fly. In parts of California and Kansas where the insect was the most important pest of wheat ten years ago, it is now almost impossible to find a single individual because of the growing of resistant varieties of wheat. The success achieved with the hessian fly can be repeated with a number of other insects. As a group, aphids offer excellent possibilities for research along these lines.

In the literature there are more than 25 records of plant resistance to different species of aphids (Painter, 1951). At Kansas State College, 6 aphid-host relationships have been studied (Table II). The 25 records of resistance to aphids represents far more cases of insect

TABLE II—Resistance to Aphids in Crops Studied at Kansas State College by Departments of Entomology and Agronomy and U.S. Department of Agriculture.

Common Name	Scientific Name	Crop	Source of Resistance	Chief Form of Resistance Studied
Pea aphid	<i>Macrosiphum pisi</i> (Har.)	Alfalfa	Ladak, Individual plants, C 104	Antibiosis, Tolerance
Spotted alfalfa aphid	<i>Therioaphis maculata</i> (Buckton), or <i>Pterocallidium</i> sp.	Alfalfa	Lahontan, Individual plants	Tolerance. Antibiosis
Greenbug	<i>Toxoptera graminum</i> (Rond.)	Barley	Dicktoo	Tolerance
Greenbug	<i>Toxoptera graminum</i> (Rond.)	Wheat	Dickinson selection from CI 3707	Tolerance
Corn leaf aphid	<i>Rhopalosiphum maidis</i> (Fitch)	Corn	K 148, K 150, WF 9, and other inbreds	Preference, Antibiosis
Corn leaf aphid	<i>Rhopalosiphum maidis</i> (Fitch)	Sorghum	Individual plant, Sudan 428-1	Antibiosis, Preference

resistance than are known in any other group of insects. At first, one might think that the close insect-plant relationship was the reason for this large number. Aphids have often been thought of as plant parasites. Their relation to the plant host is often an obligatory one. A study of the facts does not support the conclusion that resistance is more likely to develop to insects of more restricted food plants. Examples of aphids for which resistance has been found are cited in Table III, together with the number of host plants reported by Patch (1938). The numbers of host species range from 10 to 280, hence resistance to the aphid has developed in hosts of aphids with both restricted and wide host range. A consideration of cases of resistance to plant feeding insects in general shows that this lack of relationship to number of hosts is of general applicability.

This discussion will be limited primarily to the practical use of resistance for insect control and for increase in yield. Methods of discovering and utilizing sources of resistance will be stressed.

### THE SEARCH FOR SOURCES OF RESISTANCE

The first step in any study of aphid resistance is a search for sources of resistance among the host plants. To do this one must be able to study large numbers of plants of many

<sup>2</sup>The following agronomists have been especially helpful in various phases of these studies: A. J. Casady, E. G. Heyne, and E. L. Sorensen, Kansas Agricultural Experiment Station, Manhattan, and John Miller, Hays Branch Experiment Station.

<sup>3</sup>The following as graduate assistants have helped with some of the recent studies on aphids: J. J. Cartier, A. J. Howitt, David L. Matthew, E. E. Ortman, M. D. Pathak, Don C. Peters, and Emilio Viale.



TABLE III—Food Plants of Aphids for Which Resistant Varieties have been Reported.

Aphid Species	Number of Species of Host Plants*	Range of Host Plants	Where Resistance Known
<i>Amphorophora rubi</i> Kalt.	10	all in <i>Rubus</i>	<i>Rubus</i> , raspberry
<i>Macrosiphum pisi</i> (Kltb.) pea aphid	64	mostly (58) in Leguminosiae	garden peas, alfalfa
<i>Toxoptera graminum</i> (Rond.) greenbug	68	mostly (62) in Gramineae	barley, wheat, oats
<i>Rhopalosiphum maidis</i> (Fitch) corn leaf aphid	70	mostly (59) in Gramineae	corn, sorghum, barley
<i>Aphis gossypii</i> Glov. melon aphid	280	many families of Angiospermae	cantaloupe, cotton

\*According to Patch (1938).

varieties under conditions which can be held relatively constant. One advantage to aphids is that most of them can be raised in abundance and are adapted to study on plants under greenhouse conditions. Here, various factors can be kept relatively constant, and results of experiments are usually narrowly reproducible. In most cases seedling plants can be used and numbers of plants studied in the relatively small space available.

In contrast to the situation with most aphids, resistance of corn to the corn leaf aphid cannot be studied readily in the greenhouse because this aphid is unable to maintain a population on any of the corn seedlings so far tested (Viale, 1950). Therefore, resistance to this insect can be studied only in the field under outbreak conditions, or when the insect is uniformly and widely distributed. For this reason little is known about the details of resistance of corn to the corn leaf aphid. However, resistance has been studied in Illinois, Indiana, and Kansas (Painter, 1951; Viale, 1950). Table IV gives examples of the type of data that can be secured in the field. In contrast to corn, susceptible sorghum plants can be infested almost as soon as they emerge from the ground and differences in resistance at that stage are easily demonstrated.

TABLE IV—Percentage of Field Corn, *Zea mays*, Hybrids on Kansas Farms Infested by Three or More Corn Leaf Aphids, *R. maidis* (Fitch); Manhattan, Kansas, 1949 (Viale 1950).

Plant Kind	Susceptible		Resistant	
	Record No.	% infested	Record No.	% infested
Hybrids	US 523 W*	67	Kans. 1859**	4
Constituent Inbreds	Ky 27	70	K 148	0
	Ky 49	58	K 150	12
	K 55	56	N 6	29
	K 64	30	WF 9	7

\*US532W = (K55xK64) x (Ky 27xKy 49)

\*\*Kans. 1859 = (N6xWF9) x (K148xK150)

Resistant plants and strains can be sought both in the field and in the greenhouse. Often the first suggestion of resistance comes from a study of host plants under conditions of insect outbreak. Individual plants may be obviously less injured by the aphid than are their neighbors. This kind of a situation was first reported in Kansas for the pea aphid in alfalfa (Painter and Grandfield, 1935). It was seen again during the outbreak of the spotted alfalfa aphid in Kansas in 1956. Apparently resistant plants dug from farmers' fields and



studied in the greenhouse sometimes proved to be resistant to the aphids concerned. Similar studies on wheat during greenbug outbreaks have not revealed individual resistant plants.

The search for sources of resistance in the greenhouse must be adapted to the habits of the aphid and the type of resistance being studied. With the corn leaf aphid on sorghums, winged aphids will readily infest test varieties by flight, thus indicating difference in preference (Howitt and Painter, 1956). Large numbers of plants or varieties can be tested at the same time, utilizing the entire room of the greenhouse.

After infestation of the sorghums by winged forms of the corn leaf aphid, individual plants on which colonies become established may be separated from those on which no colonies develop. This gives a further screening for a second component of resistance.

This type of search has not succeeded in the case of the greenbug on wheat or barley, since the winged forms fly from the infesting material to the top of the greenhouse and very few of them come down to infest the plants under test. Yet differences are known to exist in the preference of the greenbugs for different varieties of wheat and barley (Dahms *et al.*, 1955).

In the search for resistance to the greenbug in wheat or barley and to the spotted alfalfa aphid in alfalfa, differences in tolerance have been the characteristics most easily used in screening large numbers of plants or varieties (Painter and Peters, 1956; Harvey and Hackerott, 1956). In both cases individual plants and varieties differ in their ability to support populations of the aphid studied. There is an interesting contrast in the two insect-plant relationships. While virtually 100 per cent of the plants of the barley variety, Dicktoo, have tolerance to greenbug, only about 80 per cent of the plants of Lahontan alfalfa are tolerant to the spotted alfalfa aphid. This difference between the two varieties is probably related to the genetic heterozygosity or homozygosity of the plant material.

With some aphids individual varieties such as Dicktoo barley, Lahontan alfalfa, Ladak alfalfa, and others, have been found to carry sufficient resistance for immediate farm use. With the aphids concerned, and others, individual plants appear to be the best source of resistance. This has been true of the pea aphid on alfalfa, the corn leaf aphid on sorghum, and the spotted alfalfa aphid on alfalfa. With the corn leaf aphid on sorghum and the resistant plant (428-1), a sudan type, it has been necessary to cross onto the needed agronomic types, and a long plant-breeding program is thus in prospect (Cartier and Painter, 1956). With the spotted alfalfa aphid it has been found that the well-adapted Buffalo alfalfa includes a small percentage of resistant plants (Harvey and Hackerott, 1956). It may be possible, therefore, to develop an alfalfa variety that would be resistant to the spotted alfalfa aphid and similar to Buffalo in other respects. A cooperative program to attempt this is underway at the Kansas Agricultural Experiment Station.

### THE CHARACTER OF RESISTANCE

The three components of resistance, preference or non-preference, antibiosis, and tolerance, (Painter, 1951), appear to be present to a greater or lesser degree in all cases of resistance studied. Preference has been most easily demonstrated and used in the study of the corn leaf aphid on sorghums (Howitt and Painter, 1956). It is less easily demonstrated and used in screening in other aphid-host relationships (Dahms *et al.*, 1955; Harvey and Hackerott, 1956).

Tolerance has been often the most useful kind of resistance to use in the search for sources of resistance. It is present, but virtually impossible to demonstrate, in the case of the corn leaf aphid on sorghums. In this situation the aphids cause small chlorotic spots, purple discolorations, and a certain amount of stunting. Most of these effects require large numbers of insects acting over a long period of time; therefore, tolerance to the corn leaf aphid in sorghum is not easily used in a study of a large number of varieties. In other cases of resistance to aphids, the differences found have been striking (Figs. 1 and 2).

Tolerance apparently may be developed to different types of aphid injury. Dickinson wheat differs from commonly grown varieties in that the greenbug causes less chlorosis and less stunting of the plant than in commercial wheats. The aphid resistant barley varieties, Kearney, Dicktoo, and others, are also yellowed less under greenbug attack. In studies of segregating populations involving crosses between Dickinson and susceptible winter wheat there is some evidence that tolerance to yellowing, tolerance to stunting, and the ability to survive after greenbug attack, appear separately and in various combinations in different

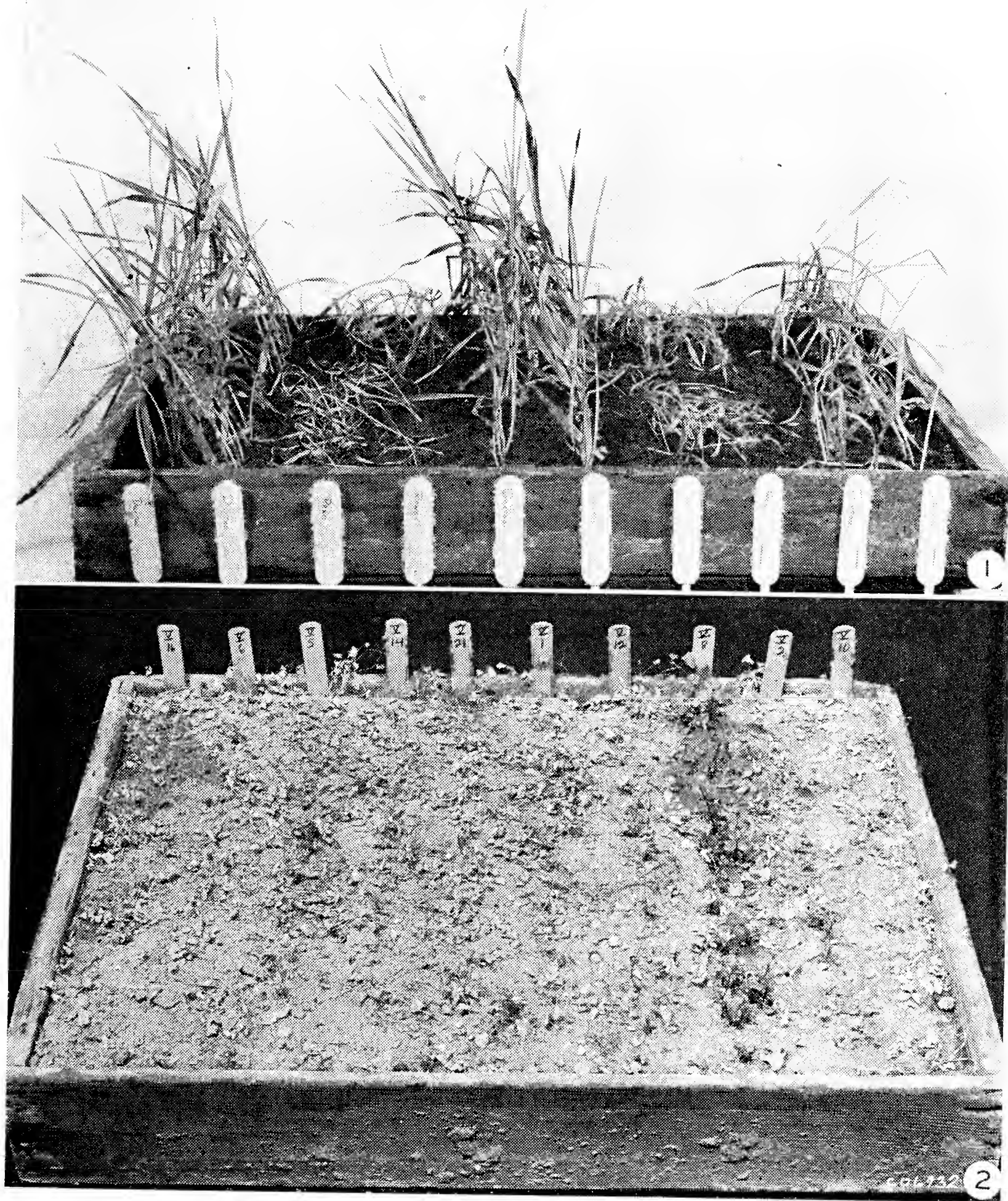


Fig. 1. Tolerance of Dicktoo barley to the greenbug in comparison with Reno barley in insectry test.

Fig. 2. Seedling resistance of Lahontan alfalfa (Row V 8) to killing by the spotted alfalfa aphid. Other named varieties shown are Ladak (Row V 5), Rhizoma (Row V 1), and Ranger (Row V 2). Greenhouse test by Sorensen, Peters, and Painter, Manhattan, Kansas, 1955-1956.

F<sub>3</sub> lines. The degree of tolerance to greenbugs so far found has been appreciably higher in barley than in wheat. In both cases it is certainly of economic importance.

The spotted alfalfa aphid produces some chlorosis in susceptible plants and somewhat less in resistant ones. The stunting produced in the field by the spotted alfalfa aphid and also by the pea aphid is quite marked in susceptible plants and much less in resistant ones. Possibly the most striking difference in all three cases has been the way in which resistant plants survived while susceptible ones were killed (Figs. 1 and 2). There has been little evidence of chlorosis in the feeding of the pea aphid on alfalfa, but in the field the stunting of susceptible plants, in contrast to resistant ones, has been marked (Painter, 1951; Fig. 14).



The term antibiosis has been applied to various adverse effects of the resistant plants on the biology of the insect. The term describes the results which may arise from the fact that the insect will not feed, as well as cases where the food is apparently deficient or toxic. The first alternative would properly be classed as non-preference, but careful experimentation is required to distinguish between the possible causes of the biological effects. In the relation of the pea aphid and of the spotted alfalfa aphid to alfalfa and the corn leaf aphid to sorghums, the effect of the resistant plant on the insect reproductive rate has been tremendous (Dahms and Painter, 1940; Harvey and Hackerott, 1956; Howitt and Painter, 1956; Cartier and Painter, 1956). In all of these cases many of the females have not reproduced at all on resistant plants, or if they did, the nymphs lived for only a short time. The females had a much shorter life span in most cases. Certainly in the field these aphids should be unable to maintain a population on resistant plants and, even with a lower level of antibiosis, the field population would be expected to decrease. If a majority of the fields in an area contained these resistant plants, one might expect the general aphid population in the area to decrease.

In the resistance of barley and wheat to greenbugs, antibiosis has been present but less useful in the search for the sources of resistance. The reproduction of the greenbug on resistant barley and wheat varieties has been only about half that on susceptible varieties (Anonymous, 1955; Dahms *et al.*, 1955; Peters, 1955). In contrast to the near immunity found to the pea aphid, the corn leaf aphid, and the spotted alfalfa aphid, the difference in antibiosis of the greenbug on the resistant barley and wheat has been of less use in the search for sources of resistance and in screening segregating populations of plants. On a field scale, however, it probably will be of economic value. Greenbug infestations occur over thousands of acres, with millions of aphids present. Under these conditions a difference in reproductive rate of half an aphid a day may make the difference between a small or mediocre yield and complete crop destruction. This antibiotic difference between resistant and susceptible varieties, even when small, reinforces the value of tolerance. In the field this small difference in greenbug reproduction on resistant and susceptible varieties would permit parasites and predators to accomplish more of their useful work on aphids on resistant varieties before severe injury to the crop occurred. In contrast, when the level of antibiosis has been high there is sometimes difficulty in demonstrating tolerance. In such cases tolerance may be demonstrated under overwhelming attack of the insect; it may be found in different plants; or the two components of resistance may be separated in segregating plant populations.

### INHERITANCE OF RESISTANCE

The inheritance of resistance to the pea aphid in alfalfa has been studied in California (Jones *et al.*, 1950), where a dominant gene linked with a recessive gene appeared to be primarily responsible for resistance in the one resistant plant studied. The genetic formula given was not entirely satisfactory however.

The inheritance of the tolerance component of resistance of barley to the greenbug has been studied in Oklahoma where differences involving one or two dominant genes for resistance have been found, depending on the particular crosses and parents involved (Dahms *et al.*, 1955).

Resistance of wheat to the greenbug, involving crosses between Dickinson selection and three winter wheat varieties, has been studied in Kansas. The  $F_2$  population appeared to show the presence of a single recessive gene for the tolerance component of resistance as expressed in ability to survive an infestation (Painter and Peters, 1956). However, the  $F_1$  of these crosses appeared to be more like the resistant parent in the lack of chlorosis following aphid feeding, and in  $F_3$  there may be a differing segregation for various expressions or kinds of tolerance. Resistance to the corn leaf aphid in the sudan plant (428-1) appeared to be dominant and carried in the heterozygous condition in the resistant plant isolated, since some of the selfed progeny from this plant have been fairly susceptible. So far, it has been impossible to isolate a homozygous resistant strain derived from this plant. In studies of resistance of corn to the corn leaf aphid the genetics of resistance appears to be complex, though the inheritance of resistance has been clear in most studies (Painter, 1951).

### ENVIRONMENTAL INFLUENCE ON THE EXPRESSION OF RESISTANCE

Repeated attempts to maintain a population of the corn leaf aphid on corn seedlings less than 30 days old have been uniformly unsuccessful. This was reported many years ago

by Forbes (1905), but the fact has been obscured by the common observation that the corn leaf aphid feeds conspicuously on the young whorl tissues of older plants. Ordinarily, resistance to insects has been greater in older plants than in seedlings, but as with the corn leaf aphid, younger corn plants are more resistant to the European corn borer than are older ones. Also, chinch bug-resistant sorghums show a higher level of antibiosis when young than when old.

There have been appreciable differences in the rate of reproduction of the pea aphid on alfalfa and of the corn leaf aphid on sorghums when reared on vegetative and fruiting parts of the same plant (Dahms and Painter, 1940; Cartier and Painter, 1956). In the case of the pea aphid and alfalfa this difference, according to the tissue on which the insect fed, was superimposed on sometimes greater differences in resistance. In some resistant plants the difference between the reproduction on the two tissues was more than 20 times as much as it was in other resistant or susceptible plants (Dahms and Painter, 1940).

Temperature also influences the expression of resistance. The reproduction of the pea aphid on resistant alfalfa plants was sometimes greater at lower temperatures, although individual plants differed in this respect. Some showed very little range of reproduction at different temperatures (Dahms and Painter, 1940). General observation in the study of the resistance of wheat to greenbugs indicates that the degree of resistance is less at high temperatures than at low. It is fortunate that the relationship is in this direction, since greenbugs typically cause damage during the early spring and late fall when temperatures are low and the expression of resistance is the greatest.

Biological strains of aphids have been found in three series of studies. Harrington (1945) reported the presence of biological races of the pea aphid in relation to resistance in garden peas. Dahms (1948) reported the presence of greenbug biotypes in his studies of resistance of barley and oats to this insect. The presence of at least two corn leaf aphid biotypes has been reported by Cartier and Painter (1956). The presence of insect biotypes emphasizes the importance of finding as many sources of resistance as possible. By so doing it may be possible to find plants with greater levels of resistance to all biotypes. It is important to look for such biotypes although in none of the cases reported have the biological strains of the species completely overcome the resistance now known.

## ECONOMIC IMPORTANCE OF APHID RESISTANCE

Three varieties resistant to aphids have been approved for use by Kansas farmers by the Kansas Agricultural Experiment Station. Ladak alfalfa, which carries moderate resistance to the pea aphid, was approved in 1937. The Kansas corn hybrid No. 1859, which carries high resistance to the corn leaf aphid, was approved in 1950. General observations would indicate that this hybrid tends to have a relatively higher yield than other hybrids in years when the aphid is abundant. It has been fairly high yielding in most years. The barley variety, Dicktoo, was approved in 1952. Dicktoo barley was developed by the North Dakota Experiment Station and had outstanding winter hardiness. Apparently its resistance to the greenbug was not known until it was studied in Oklahoma and Texas (Dahms *et al.*, 1955; Anonymous, 1955).

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### DISCUSSION

G. J. SPENCER. Does the development of resistant varieties reduce the desirable qualities of the crop?

R. H. PAINTER. Not necessarily. All varieties distributed to Kansas Farmers must be approved by all experiment station workers who deal with the particular crop. A new variety must be as good as varieties then grown in most of the important characters, and better in one or more characters, if it is to be approved for distribution. All 9 insect-resistant varieties approved by the Kansas Agricultural Experiment station have passed this test.

S. E. FLANDERS. What is the probability that aphids will become adapted to resistant varieties of host plants?

R. H. PAINTER. Adaptation to resistant varieties by an insect depends on various factors, especially complexity of the basis of resistance and the heterogeneity of the aphid germ plasm. Biotypes of hessian fly able to feed on resistant wheat varieties have not increased as expected. There is no detailed information on the adaptation of aphids.

F. G. HOLDAWAY. This is a very interesting and valuable contribution. It is my opinion that the reduction of insect injury by resistance is an approach that will be used to an increasing extent in the future.

At the University of Minnesota we have a team of corn breeders and entomologists working to reduce the losses from European corn borer by developing double cross varieties of field corn. To date four new double cross varieties that are resistant to the corn borer have been developed. During the past few years, when abundance of the corn borer has been comparatively low, these resistant varieties have outyielded the susceptible adapted varieties by an average of four bushels per acre. We are donating time and attention to the study of the mechanism of resistance.

Recently a species of aphid somewhat like the spotted alfalfa aphid has become a serious pest of sweet clover. It, together with the sweet clover weevil, *Sitona cylindricollis*, is making the growing of sweet clover a difficult problem. The University of Minnesota plans to start a programme of breeding sweet clover varieties resistant to the sweet clover weevil and the sweet clover aphid in the near future.



# Oviposition and Survival of the Wheat Stem Sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae), in Various Hosts

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## ABSTRACT

All the spring wheats that were exposed at suitable stages of growth to adequate densities of the wheat stem sawfly, *Cephus cinctus* Nort., were infested in excess of 90 per cent. Differences in infestations appeared to result mainly from differences in the rates of development of the host varieties. Increased oviposition was related to increased stem diameter, internode length, and number of elongated internodes of the host at the time of the sawfly flight.

Oviposition site is determined by host maturity. As the ovipositing female usually selects the upper internode that is elongated at the time of oviposition, most oviposition occurs in progressively higher internodes as the stem develops and flight progresses. When stems differ in maturity, heaviest infestation will occur in higher internodes in the older stems than in the younger stems; the second and third internodes from the base of the stem generally receive most of the eggs. Most oviposition is limited to the upper part of each internode. Stems in the shot-blade to boot stages are preferred for oviposition; those that are headed are seldom infested. When plants are seeded on different dates in the same area, infestations occur first in those seeded first, then progressively later in the plants seeded on progressively later dates.

Most eggs laid in the pith-cavity will hatch if not destroyed by sawfly larvae or parasites. Many eggs laid in the pith will fail to hatch; the percentage of mortality depends on the variety and the environment of the host.

Many larvae from eggs laid in the pith die in the first instar, while most of those from eggs laid in the pith-cavity become established if not destroyed by other sawfly larvae. The amount of solidness in the lower three internodes limits the survival of established larvae in the bread wheats; this relationship is less apparent in the durum wheats. Resistance of the pith to the larvae appears to be mechanical.

Various fertilizers containing nitrogen and phosphorus had no apparent effect on the resistance of Chinook, a solid-stemmed variety.

## DISCUSSION

J. VAN DEN BRANDE. I have been studying the wheat stem sawfly (*Cephus pygmaeus*) in Europe. Infestation was as high as 26% even when there was no loss in corn and the infested stems were the largest ones.

N. D. HOLMES. The larger stems produce longer heads with higher yields. Consequently the yield from these stems cannot be compared with the yield from smaller stems.

S. E. FLANDERS. Is the reproduction of the sawfly biparental or uniparental?

N. D. HOLMES. Occasional females produce females uniparentally, but most reproduction is biparental.

R. H. PAINTER. An important contribution of the Canadian workers has been the use of resistant varieties in which the resistance is varied by environmental conditions. Would you comment on the economic value of resistant varieties?

N. D. HOLMES. Annual losses from wheat stem sawfly in Canada have reached 50 million bushels. Where farmers have grown Rescue wheat the losses have been reduced to almost zero.



# Variedades de Frijol Resistentes al Picudo del Ejote *Apion godmani* Wag. (Curculionidae) y a la Mosca de las Semillas *Hylemia cilicrura* (Rondani) (Anthomyiidae) en el Valle de México

Por JOSÉ GUEVARA C.

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## ABSTRACT

*A few bean varieties resistant to the Apion pod weevil, Apion godmani Wag., have been selected from large collections and some of these are already in commercial production.*

*Many crosses have been made, some of them interspecific between Phaseolus vulgaris and Phaseolus multiflorous. Those lines in the F5 and F6 showing resistance are being compared in yield tests.*

*No correlation was found between morphological characteristics of the plants, pH of the pods and insect resistance.*

*In a preliminary study of resistance of bean varieties to the seed corn maggot a correlation was found between color of grains and resistance to the insects. The order of resistance found in 12 varieties (two of each color) was as follows: black, pinto, yellow, white and tan. It is possible that these colors are associated with some other characteristics of the seeds such as odor and taste.*

El picudo del ejote *Apion godmani* Wag. es una plaga importante del frijol en las tierras altas, arriba de 1000 metros de altura sobre el nivel del mar, no ocurre en las planicies bajas del norte del País que colindan con los Estados Unidos de América. El ataque de esta plaga está limitado a las siembras hechas durante la temporada de lluvias (mayo-septiembre). Las hembras depositan sus huevecillos en las vainas tiernas y los picudos terminan su ciclo al madurar las vainas.

La mosca de las semillas *Hylemia cilicrura* es una plaga del frijol especialmente en la temporada seca en los frijoles sembrados bajo riego. Las hembras depositan sus huevecillos desde el momento de la siembra y continúan ovipositando por algunos días en el suelo y posteriormente cerca o en las pequeñas plantitas, en la parte del hipocotilo que permanece enterrada. Las larvas generalmente cortan la plúmula de las semillas y se alimentan de los cotiledones.

El trabajo sobre variedades resistentes al picudo del ejote se comenzó hace 5 años en la Estación Experimental El Horno, Chapingo, México, bajo el patrocinio de la Fundación Rockefeller y ha constituido fundamentalmente en el siguiente desarrollo:

### 1. SELECCION DE VARIEDADES

McKelvey y colaboradores, 1951 reportaron las primeras variedades de frijol con apreciable resistencia del picudo del ejote. Durante los años 1950-55 se hicieron selecciones en mas de 400 variedades y muchos cientos de líneas; de estas selecciones, algunas variedades son las mismas recomendadas por su alto rendimiento como Roc. 1, Hgo. 12A-1, etc. y muchas otras solamente pueden utilizarse como material de cruzamiento.

### 2. ESTUDIO PRELIMINAR PARA INVESTIGAR LAS CAUSAS DE LA RESISTENCIA

En 1951 se realizó un experimento de campo con el objeto de investigar si existía alguna pseudo-resistencia debida a la diferencia en fecha de floración de diferentes variedades. El experimento consistió en sembrar 4 variedades de ciclo precoz y 4 de ciclo intermedio con 8 repeticiones, de tal manera que coincidiera su fecha de floración para permitir que el picudo del ejote atacara a todas las variedades al mismo tiempo. Los resultados mostraron que no existe pseudo-resistencia o escape en las variedades seleccionadas.

En 1952, se llevó a cabo otro experimento para observar la relación entre las características morfológicas de la planta principalmente y su resistencia al picudo del ejote. Se sembraron variedades precoces, semi-precoces e intermedias con diferente hábito de crecimiento y además con resistencia y susceptibilidad conocida. Las variedades se sembraron en

parcelas al azar con 4 repeticiones, haciendo siembras escalonadas cada 15 días. Además de los recuentos de insectos por vaina en cada parcela, se registraron los datos de hábito de crecimiento, precocidad, número de hojas, número de vainas de 1–3 cm., pubescencia de las vainas, pH de las vainas, color de flores, granos atacados y ataque de enfermedades. La comparación de todos estos datos no mostró relación alguna con la resistencia de las variedades observadas al picudo del ejote.

### 3. CRUZAS

Se hicieron cruzas entre ayocotes (*Phaseolus multiflorus*) y frijoles comunes (*Phaseolus vulgaris*) porque los ayocotes son inmunes al ataque del picudo del ejote. Las cruzas entre estas dos especies son difíciles de obtener presentándose en la segregación diversos genes letales. El mayor número de cruzas fué hecho entre las variedades resistentes al picudo del ejote y las variedades comerciales susceptibles o moderadamente resistentes con alto rendimiento, cuando se combaten las plagas con insecticida. Las selecciones de estas cruzas en F-4 y F-5 están siendo probadas en ensayos de rendimiento. Entre los problemas importantes en relación con el cruzamiento y selección de variedades de frijol para grano, está la extensa segregación que se obtiene entre algunas cruzas. Esto puede ser ilustrado perfectamente con la cruz Roc. 3 por Hgo. 6 en la cual se obtuvieron 21 colores diferentes.

En los experimentos con la mosca de las semillas, 12 variedades fueron agrupadas por color de grano. Dos experimentos iguales en cuadro latino modificado fueron analizados. Hay diferencias del 1% en los frijoles negros y pintos y diferencias del 5% para Negros, pintos y amarillos como puede observarse en la tabla No. I.

TABLA I—Promedios de Infestación de Larvas de *Hylemia cilicrura* en Diferentes Variedades de Frijol Agrupadas por Colores.

Color	Promedio
Negro brillante.	26.283**
Bayo bola.	36.500
Ayocote blanco.	31.333
Amarillo brillante.	30.266*
Pinto café	26.533**
Canario.	31.833

\*D.M.S. 5%

\*\*D.M.S. 1%

Estos experimentos fueron sembrados a mano, por esta razón el color del grano pudiera tener cierta influencia en la oviposición, pero en las siembras hechas a máquina los insectos no se guían por el color de la semilla y muy probablemente responden a diferencias en olor de los granos.

### REFERENCIA

McKelvey, J. J., Arthur C. Smith, José Guevara y Alfonso Cortés. 1951. Biología y control de los picudos del género *Apion* que atacan al frijol en México. Oficina de Estudios Especiales, S.A.G. México. Folléto Técnico No. 8.

# Which Grasshoppers of Temperate North America are Migratory; Notes on their Migratory Behavior and Their Names (Orthoptera: Acrididae)

By ASHLEY B. GURNEY

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## ABSTRACT<sup>1</sup>

Since the grasshopper problem during recent years has involved migratory species much less in temperate North America than it has in certain other continents, the occurrence and nature of the migratory habits of several species which inhabit the United States and Canada are not fully understood by all entomologists, especially those living abroad who are accustomed to apply the name "locusts" to migratory grasshoppers. Recently, several changes in the names and taxonomic rank of these grasshoppers have occurred.

The outstanding historical example is *Melanoplus spretus* (Walsh), which was notably migratory and destructive during the 1870's. A major source of confusion has been the uncertainty as to whether *spretus* was a phase of *Melanoplus mexicanus* (Saussure). The specific name *mexicanus* is now restricted to a species dominant in Mexico, and other specific and subspecific entities of the complex, each an important or potentially important pest, are recognized. These changes result from basic studies in progress, by the author in collaboration with A. R. Brooks of Saskatoon, Canada, which utilize the distinctive features of the aedeagus. It appears that *spretus* is a distinct species, and that opinion is adopted as a working hypothesis pending the acquisition of more evidence. It seems probable that the historic, devastating swarms of *spretus* primarily represented a gregarious phase. Though no solitary phase is known, one probably existed, and, if so, it may still live in localized spots.

With the discovery that *Melanoplus mexicanus* is a distinct species largely restricted to Mexico, another name is required for the widespread, variable species, previously called *mexicanus*, which inhabits nearly all of the United States and much of Canada. *M. bilituratus* (F. Walker) 1870 is the available name here adopted. During certain outbreak periods, extensive migratory flights of *M. bilituratus bilituratus* have occurred.

Observations made in the Great Basin, chiefly in Nevada and eastern Oregon, during and since the 1939-51 outbreak of *Melanoplus rugglesi* Gurney, demonstrate quite well the occurrence of gregarious and solitary phases in this species. Most of the information on phases is still unpublished.

A fourth migratory grasshopper is *Dissosteira longipennis* (Thomas), which has its natural habitat in the central area of the High Plains, mainly in eastern New Mexico, eastern Colorado, and the northern panhandle of Texas. Over a period of 85 years this grasshopper has been recorded in outbreak proportions only 4 times, especially in 1934-40.

Some migratory behavior is shown by one or more species of *Camnula*, *Oedaleonotus*, and *Schistocerca*, and there is need for more critical study from the standpoint of migrations.

## DISCUSSION

G. J. SPENCER. Are there any evidences of solitary *spretus* now existing?

A. B. GURNEY. No direct evidence is available, but by inference it seems possible.

B. P. UVAROV. I find it hard to believe that *M. spretus* is extinct. There are differences in aedeagus between subspecies of *M. mexicanus*, presumably due to environmental factors; therefore, it is not impossible that differences in copulatory apparatus are present due to changes in density. Not only the aedeagus, but other parts of the copulatory apparatus must be studied.

The paper by Dr. Gurney is a fine illustration of the need for taxonomic studies even on the so-called "well-known" species.

G. J. SPENCER. I would like to ask Mr. Putnam if he accepts the term "migration" in connection with the movements in distance of certain British Columbia species.

<sup>1</sup> For complete text see the revision by A. B. Gurney and A. R. Brooks in the *Proceedings of the U.S. National Museum*. (In press).



L. G. PUTNAM. Yes, but I do not feel sure that the term "swarm" can be applied to the grasshoppers involved in these movements, not in the sense used for old-world locusts.

R. H. PAINTER. About 1938 my wife and I drove for 15 to 20 miles through a "swarm" of *longipennis* in eastern Colorado. Insects were present as far as one could see. I would have called this a swarm.

A. B. GURNEY. The questions asked point up the value of exchanging ideas from both sides of the Atlantic Ocean.

# Movement and Direction of Swarms of the Desert Locust (*Schistocerca gregaria* Forsk.) in Uttar Pradesh (India) During 1954

By A. S. SRIVASTAVA

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## ABSTRACT

*An unprecedented invasion of the desert locust occurred in the State of Uttar Pradesh in 1954. Egg-laying was reported from more than 66,843 acres in the 16 western districts. Seven waves of locust swarms entered the State in quick succession, invading all 51 districts. The details of movement and direction of the swarms are presented.*

*Data on the periods of egg-hatching in different months in different districts are given.*

The State of Uttar Pradesh experienced an unprecedented invasion of the desert locust, *Schistocerca gregaria* Forsk., during 1954. This resulted in invasion of all 51 districts of the State, and egg-laying in more than 66,843 acres in the 16 western districts. In all, there were seven successive waves of locust swarms in quick succession. Early in 1954 reports of locust breeding were received from neighbouring countries including Saudi Arabia, Eritria and the Sudan. The locust situation at that time in Iran, Arabia, and eastern parts of Africa was also grave.

The details of movement and direction of the locust swarms are given in Figs. 1-10. The first wave was observed on May 17-18 and again on May 20-21, when a yellow swarm of locusts,  $1\frac{1}{2} \times \frac{1}{2}$  miles, visited Dehradun district and settled at two places for periods varying from 16 to 18 hours, on fallow and cultivated lands (Fig. 1). Swarms of the second wave appeared on June 21 and were present until July 17. Locusts of this wave invaded 46 districts of the State in the month of June alone. Figs. 2-4 show that the locusts invaded all but the northernmost hill districts. The third locust wave (Figs. 5 and 6), July 22—August 16, entered the State from the Delhi side and settled in practically all 51 districts. The sizes of the swarms varied from 20,000 square yards to about 21 square miles. Egg-laying was reported in 14 western districts. The fourth wave (Fig. 7), August 22—September 3, was composed of eight swarms. These entered the State from the west, and visited 21 districts. The fifth wave (Fig. 8), September 10—21, invaded the districts of Hamirpur, Jalaun, and Jhansi. Egg-laying was reported from Agra district. The sixth wave (Fig. 9), October 22—November 10, was composed of seven swarms which invaded the State from the west and south, visiting 10 districts. Egg-laying was reported in the districts of Bulandshahr, Aligarh, Meerut, Muzaffarnagar, and Saharanpur. The final wave (Fig. 10), December 23—31, invaded the districts of Mathura and Aligarh.

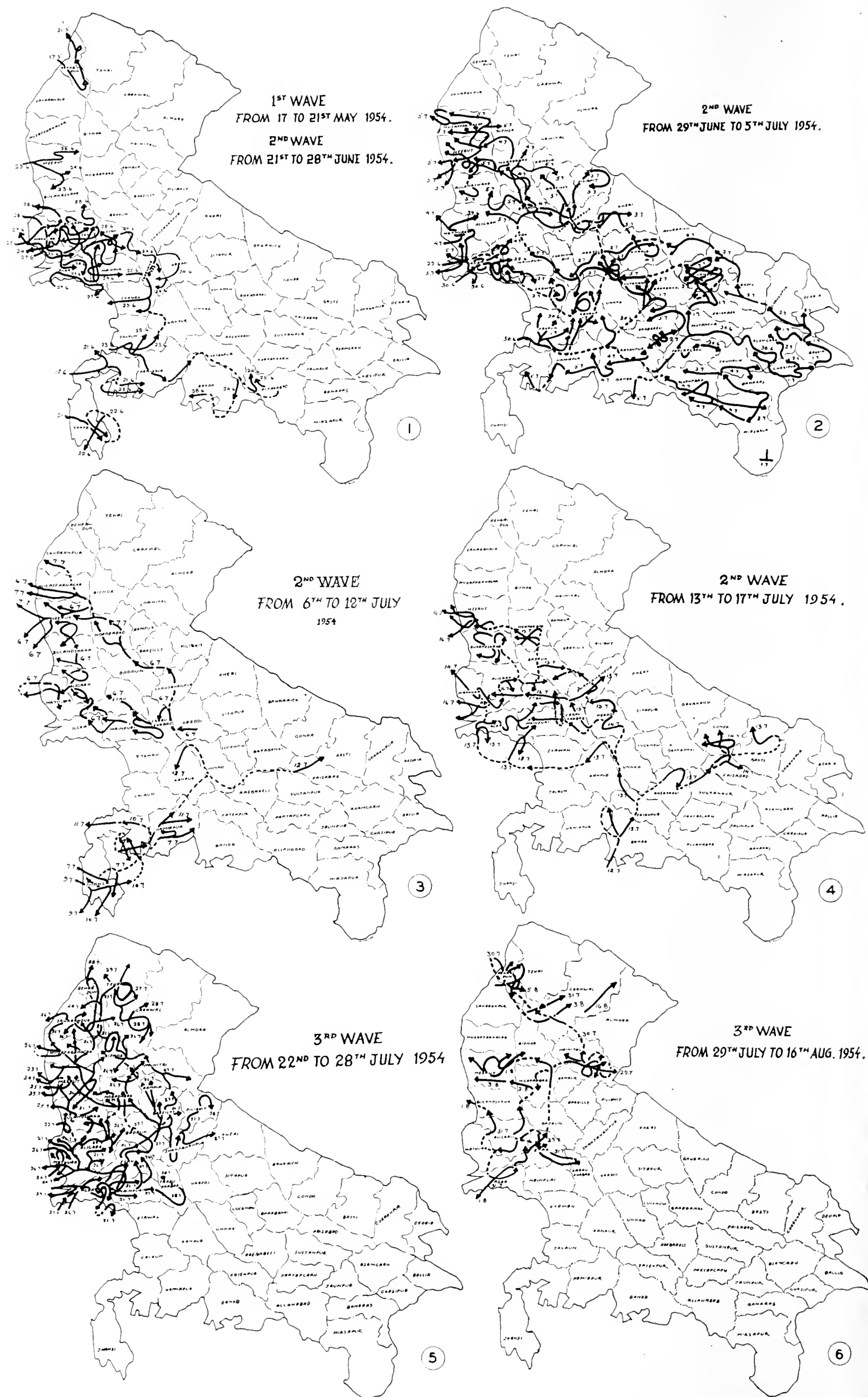
In all, about 57 swarms entered the State, mostly from the west and south-west.

Data collected regarding the period of egg-hatching in different months in different districts are presented in Table I. These show that the hatching period varied from 11-12 days to 16-20 days in July, and from 13-23 days to 17-30 days in October, the shortest periods being in the warmest districts in each case. The cold season affected emergence adversely. It delayed or prevented emergence, and fungous diseases and predators took a heavy toll.

The State Plant Protection Service went into operation immediately upon receiving word of the locust invasion, and rushed men and material from various centres and sub-centres to the affected areas. As a result of these unprecedented operations, an area of more than 136,265 acres infested by eggs and locusts was cleared by December, 1954. The damage done to crops was very small, amounting to only Rs. 34,613/8—(about \$7000) in the whole State.

## ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. S. B. Singh, Director of Agriculture, Uttar Pradesh, Lucknow, for his interest in this work.



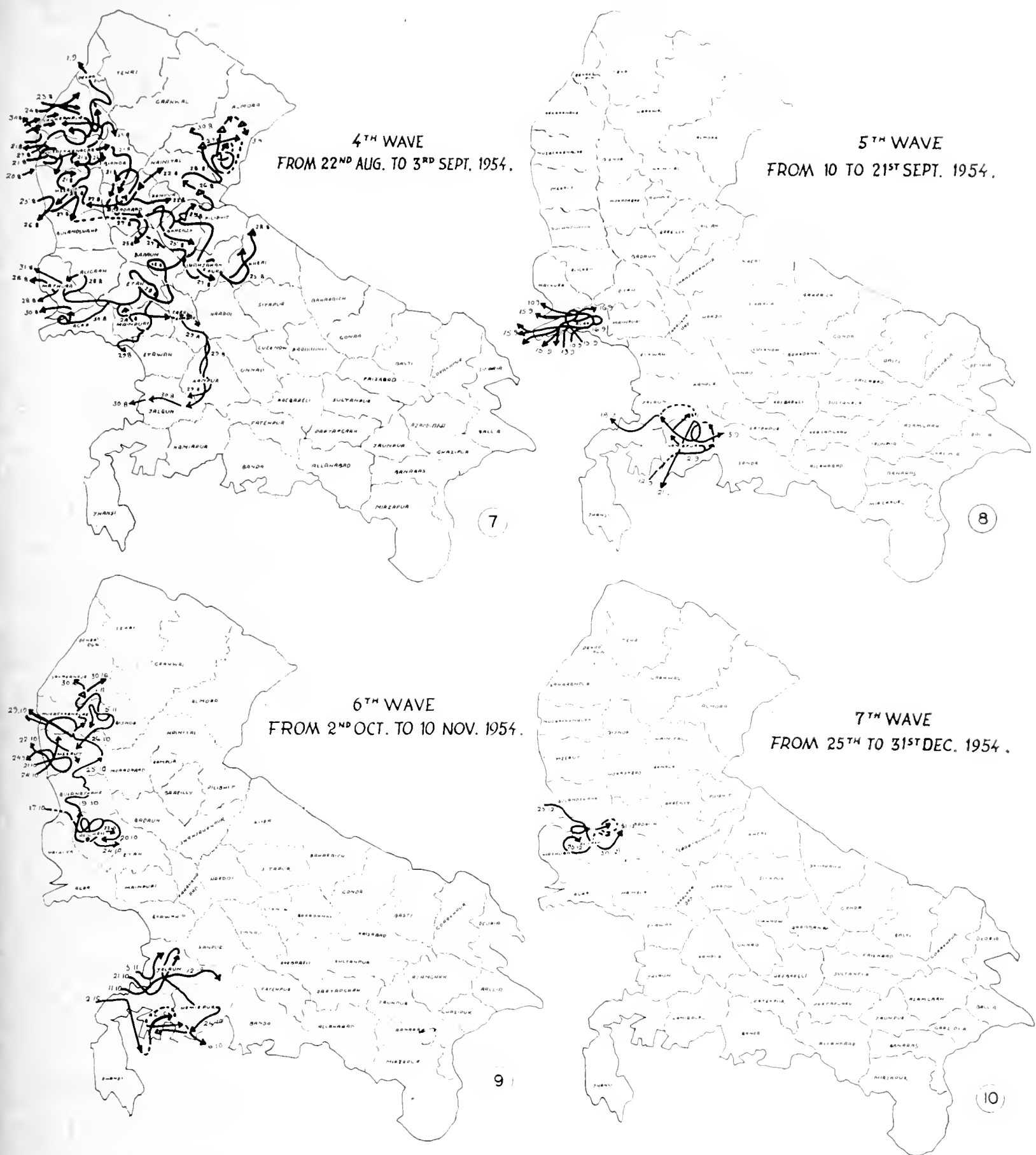


TABLE I. Incubation Period, in Days, of Eggs of the Desert Locust, Uttar Pradesh, India, 1954.

District	Month				
	June	July	August	September	October
Aligarh	—	13-16 days	—	—	21-26 days
Agra	—	13-16 days	14-19 days	16 days	—
Nainital	—	15-16 days	—	—	—
Rampur	—	14-15 days	—	—	—
Jhansi	—	14 days	—	—	—
Etah	—	14 days	—	—	—
Saharanpur	—	14-15 days	—	—	—
Mathura	—	12-14 days	13 days	—	—
Badaun	—	14-17 days	—	—	—
Pilibhit	—	16-20 days	—	—	—
Bijnor	—	18 days	—	—	—
Dehradun	—	—	—	—	—
Meerut	12-13 days	11-12 days	11 days	—	13-23 days
Muzaffarnagar	—	11-13 days	—	—	—
Bulandshahr	—	11-12 days	—	—	17-36 days
Moradabad	—	13-15 days	—	—	—





# Biología y Control de las "Tucuras" (Orth. Acrid.) en la Provincia de Buenos Aires, Republica Argentina

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## ABSTRACT

Information is given on the important losses caused in the Argentine Republic by "tucuras" (Acridian species similar to the grasshoppers of the U.S.A.) delimitating the area now invaded and describing the damage caused in fields and crops, estimated at over 1000 million pesos annually.

The species predominant in the Province of Buenos Aires are described, together with their biology and the influence of climate on their different stages of development.

Account is given of the methods employed to control them, such as dusting, poisoned bait and the more recent tendency toward spraying with emulsions and solutions with residual toxic effect, especially Heptachlor and Dieldrin, together with report of experiments with different methods of application of these insecticides.

An outline is given of the future plan of control, based on the organization of an efficient and detailed egg-laying survey, which will permit the demarcation of the infested areas and their treatment with these insecticides at the time of hatching.

Finally the author describes permanent studies to be made in areas of high infestation and of varied ecological conditions, which will permit a determination of the influences of climatic conditions, soil characteristics, predominant plant cover, etc., on the embryonic development, data which will be of great value in the organization of chemical warfare against these acridians.

## IMPORTANCIA ECONÓMICA Y DISTRIBUCIÓN GEOGRÁFICA

Con la denominación genérica de "tucuras" (voz guaraní que significa semejante a langosta), se agrupan especies de Ortópteros, de la familia Acrídidos, caracterizados por sus hábitos sedentarios o semisedentarios, lo que hace que solo realicen cortos vuelos de desplazamiento, aoven en el área en que viven y pasen el invierno al estado de huevo en el suelo. Es decir, que su ecología es similar al de las especies conocidas en los Estados Unidos como saltamontes ("grasshoppers").

Se han citado para la República Argentina más de cien especies de "tucuras", diseminadas prácticamente a través de todo su territorio, pero solo constituyen problema de extraordinaria incidencia económica en la Provincia de Buenos Aires, parte de la Provincia de La Pampa y en la región sur de la de Córdoba. Más de 15 millones de hectáreas de la región agrícola-ganadera más importante del país son afectadas por las "tucuras".

La Provincia de Buenos Aires tiene actualmente un área infestada que se calcula no inferior a 7 millones de hectáreas, lo que significa que la producción agropecuaria del 25% de la superficie de la provincia, es esquilmada por la plaga, determinando pérdidas anuales que no es aventurado calcular, superan los 1000 millones de pesos. Tales pérdidas involucra lo que destruye—cultivos de cereales, lino, alfalfa, girasol, sorgos, etc.—y la merma de forraje en los campos de pastoreo en detrimento del engorde del ganado.

Es interesante señalar que el problema, en lo que concierne a Buenos Aires, adquiere mayor gravedad año tras año al extenderse constantemente el área de dispersión de la plaga. Este incremento, atribuido en buena parte a la notable disminución en la región de las aves insectívoras y en especial de las gaviotas, se refleja a través de los diversos trabajos publicados sobre el tema.

En el año 1927, T. Joan, refiere que la especie de "tucura" más dañina—*Dichroplus maculipennis*—localizada en principio en Choele-Choel y la región norte del Río Negro, se ha extendido a Bahía Blanca, General Lamadrid y Olavarría, alcanzando hacia el oeste hasta Santa Rosa. Veinte años más tarde, Liebermann y Schiuma, citan como partidos intensamente infestados a General Lamadrid, Olavarría, Laprida, Coronel Suarez y Coronel Pringles, en cuyos campos bajos, refieren, es común hallar más de 500 desoves por metro cuadrado. Con infestaciones menores pero suficientes para producir daños de consideración, señalan

a los partidos de Gonzalez Chaves, Tres Arroyos, Guaminí, Caseros, Tapalque, Bolivar y Pehuajó.

En la actualidad, como se observa en el mapa que se incluye, referente a la distribución geográfica de la plaga, la superficie invadida ha aumentado considerablemente, notándose el desplazamiento de la "tucura" hacia el este y noroeste de la Provincia.

### ESPECIES PREDOMINANTES Y CICLO DE VIDA

En la República Argentina se han determinado más de 300 especies de Acrídidos, agrupadas en seis subfamilias, de las cuales la más importante es la de los Cirtacantacridinos. Liebermann cita para la Provincia de Buenos Aires las siguientes especies de "tucuras":

*Dichroplus maculipennis* (Blanch.) Lieb. "La tucura de las alas manchadas".

*Dichroplus bergi* Stal. "La tucura de Berg".

*Dichroplus conspersus* (Bruner). "La tucura manchada".

*Dichroplus elongatus* G. Tos. "La tucura alargada de los alfalfares".

*Dichroplus punctulatus* (Thunberg). "La tucura punteada".

*Chromacris espiciosa* Thunberg. "La tucura del palque".

*Zoniopoda tarsata cruentata* (Blanch.). "La tucura sangrienta".

*Xyleus* spp.

*Scyllina variabilis* (Bruner). "La tucura variable".

*Staurorhetus longicornis* G. Tos. "La tucura de antenas largas".

De las señaladas, "La tucura de las alas manchadas"—*Dichroplus maculipennis*—predomina en forma neta a través de todo el territorio bonariensis infestado con la plaga y es, indudablemente, la causante de las cuantiosas pérdidas que se registran anualmente. Las demás especies no revisten importancia económica en la Provincia y solo se las encuentra esporádicamente.

El ciclo biológico de *Dichroplus maculipennis*, se inicia normalmente a mediados del mes de octubre, en que nacen las pequeñas ninfas (5 á 7 mm. de longitud). Después de 10 á 12 días se produce la primer ecdysis, midiendo las ninfas del segundo estadio entre 7 y 8 mm. Su duración es de 8 á 12 días. Estas dos formas ninfales se caracterizan por la ausencia de esbozos alares y por sus hábitos gregarios, que hacen que se desplacen poco de los sitios donde ha ocurrido el nacimiento. En estos dos primeros estadios ninfales la "tucura" es denominada vulgarmente "mosquita".

En el tercer estadio ninfal, en el cual mide alrededor de 10 mm., aparecen los esbozos alares y aumenta la movilidad como consecuencia de su mayor voracidad, comenzando a dispersarse, provocando consecuentemente mayores daños y haciéndose cada vez más dificultoso su control.

Con intervalos de 10 á 15 días experimenta dos nuevas mudas, midiendo en el 4° y 5° estadio ninfal, alrededor de 13 y 16 mm. respectivamente. En estos tres últimos estadios ninfales la "tucura" es llamada comunmente saltona. Normalmente en Buenos Aires esta etapa se cumple durante los meses de noviembre y diciembre, por lo cual la dispersión de la "tucura saltona" suele provocar gravísimos daños en los cultivos, particularmente en años de sequía, en los que la escasés de pastos naturales determinan un mayor desplazamiento del acrídido en busca de alimento.

A fines de diciembre, normalmente, la "tucura" alcanza su estado adulto, en el que mide de 22 á 23 mm. La "voladora", denominación con que se distingue en el medio rural al imago, forma mangas que realizan vuelos a veces relativamente extensos. Schiuma cita haber comprobado recorridos hasta de 12 kilómetros, a una altura media de 10 metros.

Los daños que provoca la "tucura" adulta son considerables. Arrasa alfalfares, cultivos de maíz, girasol, sorgo y las siembras tempranas de cereales, destinadas a pastoreo.

Pocos días después de haber completado su desarrollo los adultos se acoplan y comienza la aovación. Cada hembra aova varias veces, haciéndolo generalmente en los bajos de los campos naturales. Cada desove contiene de 25 á 40 huevos, de color amarillo, de 3,5 á 4,5 mm. de largo. La profundidad a que son colocados los desoves varía entre 1 y 4 centímetros.

Terminado el período de ovación los adultos mueren, cerrándose así el ciclo. El huevo, forma en que la especie sortea el período invernal, eclosiona en la primavera siguiente, reiniciándose el ciclo.

El enunciado, constituye el ciclo normal de la especie que muchas veces experimenta variaciones sensibles, condicionadas a los factores ambientales. Así por ejemplo, el nacimiento de la "tucura" requiere que se produzcan temperaturas máximas, diarias, superiores a 20° C. durante varios días. Condición que puede anticipar o retrasar considerablemente la época de iniciación de los nacimientos.

La altitud, naturaleza del suelo, cobertura de la superficie del mismo por agua o vegetación, determina por otra parte, que aún en una misma zona se produzcan nacimientos con diferencias de muchas semanas entre sí. El autor ha tenido oportunidad de observar en el Partido de General Lamadrid, al promediar el mes de diciembre, en un mismo campo, "tucuras" en todos los estadios ninfales conjuntamente con los primeros adultos de la generación.

Las variaciones apuntadas con respecto a las fechas de eclosión de los desoves, ha determinado que se atribuya a *Dichroplus maculipennis* más de una generación anual.

Cabe finalmente hacer una acotación en lo que se refiere al número de estadios ninfales, que en el ciclo descrito se establece en cinco, mientras que en observaciones realizadas con insectos en cautividad se han determinado seis estadios ninfales.

## PROCEDIMIENTOS DE LUCHA; SU EVOLUCIÓN. EXPERIMENTACIÓN Y CAMPAÑAS FUTURAS

Hasta el año 1937 la lucha contra las "tucuras" en la Argentina se realizó mediante el empleo de procedimientos mecánicos, físicos y culturales. El uso de barreras metálicas para la protección de cultivos amenazados por "saltonas", que hacían posible su concentración en bretes en los que eran destruidas, o el empleo de quemadores ("lanzallamas") accionados a gas oil o fuel oil, eran las onerosas prácticas usadas hasta entonces, complementadas con las araduras invernales de los campos infestados con desoves, labor esta última, siempre recomendable donde la naturaleza del suelo lo permite.

Los métodos químicos de control comienzan a usarse a partir de la campaña 1938-39, con el empleo de cebos tóxicos a base de afrecho de trigo y arsenito de sodio.

Desde 1947, a consecuencia de los ensayos realizados con hexaclorociclohexano en el Laboratorio de Acridiología de José C. Paz, se adopta este insecticida para el control de la plaga, recomendándose su uso en espolvoreos al 10% y 20% (1,2% a 2,4% de isómero gamma), o en cebos tóxicos, en reemplazo del arsenical, a razón de 300 gramos de H.C.H. por cada 100 kilogramos de afrecho.

El uso del hexaclorociclohexano señaló indudablemente un avance en el control químico de la plaga, pero debido a su escasa acción insecticida residual y a su costo elevado, su empleo fué resistido por los productores, circunscribiéndose la lucha a la realizada por el Estado, insuficiente e inorgánica, lo que determinó que el área de infestación se extendiera constantemente, con los consiguientes perjuicios para la economía de la Provincia.

La creciente gravedad del problema hizo necesaria la experimentación de otros plaguicidas y el ensayo de otros métodos de dispersión. Durante las primaveras de 1953, 1954 y 1955 el autor tuvo a su cargo la realización de trabajos experimentales a campo, para determinar la eficacia de diversos plaguicidas. En las pruebas de referencia se ensayaron Lindane, Chlordane, Aldrin, Dieldrin y Heptacloro, formulados en emulsión y en unos pocos casos, disueltos directamente en gas oil.

Las aplicaciones se hicieron mediante el empleo de los equipos aéreos y terrestres que se mencionan seguidamente:

- a) Avión "Auster" provisto con barra de 20 picos; capacidad del tanque 320 litros; altura de vuelo 3 metros; velocidad 95 kilómetros por hora; banda cubierta por pasada 15 metros; volumen de líquido dispersado por hectárea 22 á 25 litros.
- b) Pulverizadora "Mist-blower" (nebulizadora); marca "Simplex"; banda cubierta 6 á 8 metros; volumen de líquido dispersado por hectárea 40 á 60 litros;

- c) Pulverizadora de bajo volumen para herbicidas; marca "Berini"; provista de 14 picos; dispuesta sobre tractor con la barra en la parte delantera; banda cubierta por pasada 7 metros; volumen de líquido dispersado por hectárea 70 á 75 litros; velocidad 7 á 8 kilómetros por hora; presión 25 á 30 libras.
- d) Pulverizadora a mochila con pico para bajo volumen; marca "Potente"; banda cubierta por pasada 1,80 metros; volumen dispersado por hectárea 32 á 35 litros; velocidad de marcha del operador 4,5 á 5 kilómetros por hora.

Es de hacer notar que en los resultados obtenidos con los distintos plaguicidas y que se consignan sucintamente más adelante, el equipo usado para la dispersión jugó un papel secundario, siendo factor fundamental la dosis empleada por hectárea. De ello se deduce que la elección del equipo está condicionada principalmente a la extensión del área a tratar. Solo en algunas circunstancias, la pulverización de emulsiones desde el avión produjo fracasos en los resultados, motivados presumiblemente por la evaporación del agua con deficiente dispersión de la droga, provocado por excesiva altura de vuelo y temperatura ambiente elevada. Por ello es aconsejable que para pulverizaciones con avión se empleen siempre los insecticidas en vehículos aceitosos tales como el gas oil o diesel oil.

El comportamiento de los distintos compuestos, apreciado a través de los múltiples ensayos efectuados, se sintetiza a continuación:

"LINDANE": El "Lindane" emulsionable a dosis de hasta 90 gramos por hectárea dió buenos resultados para el control de la plaga, aún en su estado adulto. La intoxicación del acrídido se produce rápidamente; entre los 20 y 60 minutos de la aplicación la acción derribante se deja sentir sobre la totalidad de los individuos alcanzados por el líquido.

El "Lindane" carece prácticamente de acción residual, ya que a las 24 horas de realizados los tratamientos solo se observan "tucuras" muertas o no afectadas, lo que revela que no produce ningún efecto sobre los individuos que reinfestan la zona tratada.

"CLORDANE": Se lo aplicó a dosis de 600, 800, 1.000 y 1.100 gramos por hectárea. Se obtuvieron buenos resultados con un control que supera el 90%, empleando no menos de 800 gramos por hectárea. La aparición de los síntomas de intoxicación estuvieron correlacionados con las dosis, las mayores determinan que ocurra dentro de las dos horas de realizados los tratamientos. Con 600 gramos por hectárea recién a las 24 horas se notan individuos afectados y derribados. Hasta 6 días después de las aplicaciones, momento en que se interrumpieron las observaciones, el Clordane mantuvo su efecto insecticida en el terreno.

"ALDRIN": Este compuesto fué únicamente ensayado a la dosis de 300 gramos por hectárea. Su comportamiento fué muy bueno, percibiéndose su efecto entre las 2 y 6 horas de la aplicación. Hasta 5 días después se constató que mantenía el poder insecticida.

"DIELDRIN": El Dieldrin dió excelente resultado en todas las pruebas efectuadas. Las dosis más adecuadas se establecieron entre 150 y 200 gramos por hectárea; a la dosis mayor el efecto residual es evidentemente superior. El autor tuvo oportunidad de observar en uno de los ensayos en que se aplicaron 200 gramos por hectárea, que 28 días después del tratamiento el Dieldrin mantenía su efecto tóxico para "tucuras" adultas y ninfas de 4° y 5° estadio.

Es de hacer notar que el efecto del Dieldrin sobre la "tucura" se manifiesta después de un lapso variable entre 10 y 24 horas, según estado de desarrollo del insecto, dosis empleada y temperatura ambiente.

"HEPTACLORO": A principios del año 1955 y durante la última primavera se realizaron los primeros ensayos en la Argentina con Heptacloro, con resultados tan promisoramente exitosos, que determinaron su adopción para encarar próximas campañas de lucha.

Las dosis ensayadas variaron entre 225 y 425 gramos de producto grado técnico por hectárea (equivalentes a 162 y 306 gramos de Heptacloro puro). Se determinó que las cantidades necesarias por hectárea para obtener un buen control y persistencia de acción estaban comprendidas entre 280 y 330 gramos de Heptacloro grado técnico.

El efecto tóxico se manifiesta en las "tucuras" a los 30 minutos en las dosis mayores y a los 90 minutos con las menores. La sintomatología que acusan los individuos afectados consiste en incoordinación de movimientos, dificultad en el salto e incapacidad para volar.



A las 24 horas la mortalidad que se obtiene con ambas dosis es prácticamente del 100%. El efecto residual del Heptacloro es muy bueno; si bien la poca experimentación efectuada no ha permitido establecer fehacientemente el término durante el cual prolonga su acción en el terreno, se considera que el mismo oscila entre 15 y 25 días, según sea la dosis usada y las condiciones del ambiente.

De los insecticidas clorados ensayados se destacaron netamente, como se observa, el Dieldrin y el Heptacloro. Este último tiene a su favor el costo, sensiblemente menor que el del Dieldrin, lo que neutraliza la diferencia en la dosis requerida por hectárea para el logro de un control similar y aún acuerda ventajas al Heptacloro.

Los resultados relatados y los obtenidos por productores y empresas privadas, demostraron que es factible el control eficiente y económico de la "tucura" con plaguicidas de efecto residual, que empleados oportunamente, posibilitan la exterminación de los focos iniciales de infestación. La evidencia creó un ambiente propicio entre los productores, quienes demandaron la rápida importación de las drogas mencionadas para encarar en sus predios la lucha contra el acrídido.

En las postrimerías de la campaña 1955-56 se comenzaron a emplear en la Argentina, en escala comercial, Dieldrin y Heptacloro. Del primero fueron importados por avión 48 toneladas que se dispersaron principalmente mediante equipos aéreos y de Heptacloro, se aplicaron más de 10.000 litros formulados en emulsión.

La próxima campaña de lucha contra la "tucura" se ha planificado en base al empleo, casi exclusivo, de Heptacloro y Dieldrin. El Banco Central de la República Argentina ha otorgado divisas para la importación de 275 toneladas de Dieldrin, grado técnico, y de 225 toneladas de Heptacloro, grado técnico.

Con las cantidades anteriormente consignadas pueden tratarse aproximadamente 2.300.000 hectáreas, y tomando en consideración que ambos compuestos por su elevado poder tóxico residual pueden aplicarse en franjas de 10 á 12 metros de ancho, separadas entre sí por bandas de 20 metros, se dispondrá de droga como para brindar protección a más de 5.000.000 de hectáreas.

Como dato ilustrativo complementario se informa acerca de las cantidades aproximadas de insecticidas, empleados por los servicios oficiales, durante la campaña próxima pasada:

Hexaclorociclohexano en polvo al 10% y 20% . . . . .	8.000.000 k.
Cebos tóxicos (con hexaclorociclohexano) . . . . .	7.000.000 k.
Dieldrin (grado técnico) . . . . .	40.000 k.
Otros clorados (incluye Heptacloro y Lindane) . . . . .	20.000 l.

## ORGANIZACIÓN DE SERVICIOS PARA LOCALIZACIÓN DE DESOVES Y ESTABLECIMIENTO DE ÁREAS PERMANENTES DE ESTUDIOS ECOLÓGICOS

Paralelamente con el desarrollo de nuevas técnicas de aplicación y con el empleo de modernos plaguicidas para el control de la "tucura", se consideró indispensable proseguir investigaciones sobre la ecología de la plaga y establecer un adecuado sistema de localización de desoves, que permita encarar racionalmente la lucha, aplicar en la época más oportuna los insecticidas, haciendo más económicos los tratamientos.

Para la consecución de ese fin, el autor propuso un plan de trabajo, que con modificaciones de forma, ha sido puesto en ejecución por la Dirección de Defensa del Agro del Ministerio de Asuntos Agrarios de Buenos Aires. El mismo consiste en el establecimiento de un Servicio para el Reconocimiento de Desoves y de otro para realizar estudios ecológicos, en áreas permanentes intensamente infestadas.

Los objetivos del Servicio de Reconocimiento de Desoves consisten en determinar las superficies infestadas en predios, caminos, vías férreas, etc., a los efectos de notificar a los productores sobre la existencia de desoves en sus campos y para confeccionar los mapas que demarquen las zonas infestadas, todo lo cual permitirá encarar racionalmente la lucha primaveral.

Por otra parte la delimitación de áreas intensamente infestadas de 3 á 5 kilómetros cuadrados de extensión, ubicadas en zonas de la Provincia con distintas características



climáticas, hará factible la realización de estudios ecológicos de fundamental interés, tales como por ejemplo:

- a) Determinar densidad de desoves por unidad de superficie en las distintas regiones.
- b) Influencias de las condiciones del medio (temperatura del suelo, altitud, naturaleza del suelo, etc.) sobre el ciclo evolutivo de la plaga.
- c) Relación existente entre las especies predominantes de la flora natural con la intensidad de infestación.
- d) Epocas de nacimiento en las distintas zonas. Determinación del por ciento de viabilidad de los huevos. Estudio de los enemigos naturales.

Cada centro de estudios permanentes, ha de constituirse además, en un lugar de extensión para asesoramiento y enseñanza de los productores y como sistema de aviso de alarma para indicar la iniciación de los nacimientos en la región.

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# Penetration and Destruction of Plant Tissues During Feeding by *Lygus lineolaris* P. de B.

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## ABSTRACT

In an attempt to determine the cause of the exceptionally poor germination of seeds of the Umbelliferae, it was found that *Lygus lineolaris* P. de B. destroys the embryo without disturbing the other parts of the seed. Subsequently it was determined that this insect also greatly reduces seed yields by feeding on the ovaries and flowers.

Since *Lygus* are rather omnivorous feeders and produce worldwide damage to many different plant species, a study of the feeding mechanics was undertaken. By observing the stylets with the aid of the microscope and recording in slow motion on picture film it was found that considerable mechanical destruction occurs as the insect searches for cell nutrients in various plant tissues. The flexible needle-like stylets move rapidly in various directions, and frequently plunge, into and withdraw from, tissues without following a particular path in travelling inter- and intra-cellularly. A great deal of damage occurs as many cells are ruptured and emptied. Subsequently, cells in various stages of disorganization and large cavities are found in the necrotic lesions which develop in the feeding area.

It has not yet been determined whether these lesions are produced solely by the mechanics of piercing and sucking or whether in addition the saliva deposited during feeding produces secondary infections and/or is phytotoxic. The amounts of fluid imbibed and the quantity of oral secretions deposited in the host tissue were determined with the use of radioisotopes. These various techniques are demonstrated in a motion picture film which also illustrates the movements of the stylets and the deposition of saliva during feeding.

A problem confronting seed growers has been the production of many poorly-germinating seed crops of such commercially important members of the Umbelliferae as carrot (*Daucus carota* L.), celery (*Apium graveolens* L.), dill (*Anethum graveolens* L.), fennel (*Foeniculum dulce* Mill.), parsley (*Petroselinum hortense* Hoff.), parsnip (*Pastinaca sativa* L.), etc. In some cases as high as 50 per cent or more of a fresh crop of apparently well-filled umbelliferous seeds cannot germinate because the embryo is lacking although the endosperm appears normal.

It has been established (Flemion *et al.*, 1949) that embryolessness in this plant family is the result of infestation of the tarnished plant bug, *Lygus lineolaris* P. de B. [= *L. oblineatus* (Say)]. When either the nymphs or adults were caged and allowed to feed on the developing seed they destroyed the embryo at almost any stage of development but caused no apparent damage to either the endosperm or the fruit coats. In no way can these embryoless seeds be eliminated by external appearance, weight or size difference. In addition to being responsible for embryolessness in the Umbelliferae, *L. lineolaris* also adversely affects plant development (Flemion and MacNear, 1951) and seriously reduces seed yield (Flemion and Olson, 1950). Embryolessness has been reported in a number of other plant families but the occurrence is infrequent and quite low (0.1% or less in the cereals) when contrasted to the frequent high percentages in the Umbelliferae.

Prior to obtaining the above results regarding the destructive feeding of *L. lineolaris* various possible factors such as source, crop year, variety, seed yield, climatic conditions, genetical influence, etc. were studied, but no relationship of any of these with the occurrence of embryolessness was revealed. A survey was made of the types of insects which visited carrot, dill, and fennel growing in the field at Yonkers, New York. Various bees, wasps, flies, beetles, and bugs were listed. It was especially noted that nymphs as well as adults of the tarnished plant bug were found from the flowering period to the mature seed stage. It was then subsequently shown that *L. lineolaris* consistently causes high percentages of embryolessness when caged with umbelliferous plants. In the open field where plants were exposed to all types of insects, the percentage of embryoless seeds ranged from 0 to 62 per cent, while no embryolessness occurred in the seeds from umbels protected from insects by cages. With plants caged with *L. lineolaris* throughout the period of flowering

to the production of mature seed, the amount of embryoless seeds obtained ranged from 1 to 100 per cent, with an average of 58 per cent. There was a persistent tendency for seeds produced out-of-doors later in the season to have a higher average percentage of embryolessness. This increment in incidence of embryoless seeds as the season progresses is attributed to the population increase of the insect. That the nymphs as well as the adults cause damage is of importance since in certain seasons they are more numerous than the adults. When the various other kinds of insects found on umbelliferous plants were caged with carrot, dill, and fennel, in some cases nothing happened. In other cases the seeds were either entirely destroyed or the endosperm was attacked, the embryo being left intact (Flemion *et al.*, 1949; Flemion and Olson, 1950). It is of interest that *L. lineolaris* seeks out the embryos which comprise such a small and obscure portion of the plant exposed to the insects.

In the Umbelliferae double fertilization occurs (Jurica, 1922). The endosperm soon becomes mature and firm while the embryo slowly undergoes changes and reaches maturity at about the time the external appearance of the seed changes from green to brown. The mature embryo is quite small and lies imbedded in the endosperm, while in embryoless seed there is a cavity in the endosperm where the embryo would normally be located (Flemion and Waterbury, 1941). Since the embryo in its development remains immature over such a relatively long period of time, *L. lineolaris* has a great opportunity to feed upon and destroy it. Although they feed at random, an individual insect may attack both seeds of a given pair during one feeding period. Thus, pairs of seeds tend to be alike with regard to the absence or presence of embryos.

Members of the genus *Lygus* have been known to reduce seed yields in various other species of plants, for they are rather omnivorous feeders and produce bud-blasting, blossom and young fruit-drop, retardation of vegetative growth, general debilitation of the plant, and partial, as well as, complete destruction of the seeds. Much of the literature pertaining to seeds was reviewed earlier (Flemion *et al.*, 1949). Various investigators have shown that seed yields are greatly increased when the bugs are controlled. In the Umbelliferae not only are seed yields increased but in addition the percentage of embryolessness is considerably reduced by adequate control.

An examination of various umbelliferous seeds (Flemion and Henrickson, 1949) from Australia, Japan, Africa, Europe, Canada, and different areas in the United States, revealed that whenever embryolessness was detected some species of *Lygus* had been reported in the region where the seed was produced. While thus far only these insects have been found to produce embryolessness in this family, it is possible that other insects or perhaps some cultural condition may also produce these worthless seeds.

Plant bugs produce a tremendous amount of destruction to over 50 different economic crops as well as many native, wild, and cultivated plants (Knight, 1941). They attack such widely different plants as: sugar beet (*Beta*), *Chrysanthemum*, carrot (*Daucus*), cotton (*Gossypium*), alfalfa (*Medicago*), bean (*Phaseolus*), peach (*Prunus*), clover (*Trifolium*), guayule (*Parthenium*), etc.

Plant bugs pierce various plant tissues with their fine, sharp, needle-like stylets and suck out the juices (Awaiti and Wolfe-Barry, 1914). Since they do not transmit diseases, and the local lesions produced seem to be out of proportion to the extent of feeding, it has been suggested that some toxic material in addition to the withdrawal of cell fluids may be responsible (Baker *et al.*, 1946; Smith, 1920/1921, 1926). In studies undertaken to determine whether oral secretions are injected into host tissue, *L. lineolaris* was made highly radioactive by allowing it to feed on sucrose solutions to which radioactive phosphate had been added. On subsequent feeding on bean pods the insect imparted radioactivity to the tissue at the feeding site as shown by counting and by radioautographs (Flemion *et al.*, 1951).

By establishing that secretions are actually involved in the feeding, the results served as a logical background for further tests (Flemion *et al.*, 1952) with these secretions. Saliva was collected from insects containing large amounts of radioactive phosphorus ( $P^{32}$ ). By relating the amount of activity imparted to host tissue and the activity per unit volume of saliva of the insect concerned, an estimate of 0.05 to 0.25 microliter was obtained of the quantity of secretion deposited. Times of feeding were observed, but the amount of activity imparted was not directly associated with the length of feeding time. Apparently

the insects are not necessarily actually feeding during the whole time that the stylets are inserted into the host tissue, so that the observed times are not really true measurements of total feeding time. In regard to the amount of fluid imbibed during feeding, estimates of 0.2 to 2.0 microliters were obtained with the use of radioactive silver ( $\text{Ag}^{109}$ ) and cerium ( $\text{Ce}^{144}$ ).

Since the amount of damage to various plant tissues is not directly related to the length of feeding, studies were undertaken (Flemion *et al.*, 1954) to determine the manner of penetration and the subsequent damage of host tissue by the piercing-sucking mouth parts (stylets) of *L. lineolaris*. The movements of the fine, needle-like, chitinous stylets were observed in thin sections of bean pod, carrot and beet root tissue through the microscope. The extremely flexible stylets moved rapidly in various directions with frequent plunging and withdrawal, and appeared to be following no particular path. This irregular route could be followed more closely when sections of tissue only a few cells in thickness were taped to glass. The stylets travel in the tissue by short rapid thrusts and bend in any direction such as turning back or making a right angle. These various movements have been recorded on motion picture film.

In addition to following the various movements of the stylets it was also possible to observe with the microscope the removal of the contents of individual cells of beet root. In their search for nutrients the insects sucked up the red pigments which were later excreted. The emptying of a given cell was so extremely rapid that it was difficult to follow the actual process, but the cell before and after removal of the pigments was visible evidence that feeding had occurred.

By using an electrical apparatus (Ledbetter and Flemion, 1954) stylets *in situ* were obtained for histological studies. When penetrating the various tissues of bean pod the stylets traveled between as well as through the cells by puncturing cell walls. Sometimes the stylets took a straight path; however, the route usually followed no particular pattern. In passing through the intercellular spaces, the stylets forced the cells apart. In histological studies (Flemion *et al.*, 1954) neither feeding tracks nor any other indication except occasional holes were seen in the path taken by the stylets when traveling from the surface to the feeding area. However, the route taken in the tissue could be readily followed when stylets *in situ* were obtained.

From the structure of the mandibles and the activity of the stylets in host tissue, it is not difficult to visualize that a great deal of mechanical injury can occur during the feeding process. In addition to laceration of the tissues and removal of cell fluids, other factors such as those arising from the deposition of oral secretions or introduction of microorganisms may also contribute to tissue breakdown. Damage at the surface was usually slight in relation to internal breakdown. In the necrotic lesions which developed after feeding, many cells were affected—some had collapsed, while others were in various stages of disorganization.

A film illustrating the feeding mechanics of *L. lineolaris* has been produced at the Boyce Thompson Institute. It illustrates in slow motion the rapid movements of the stylets when in search of cell nutrients, and the actual deposition of saliva during feeding. Techniques are demonstrated which were used to determine with the aid of radioisotopes the amounts of fluids imbibed and the amounts of oral secretions deposited in the host tissue during feeding. Photomicrographs are shown, such as stylets *in situ* (intra- and intercellular) and internal tissue breakdown following feeding, that is, cavities and collapsed and plasmolyzed cells. Also included in the cinephotography are various other types of feeding damage, such as retardation of stems and leaves, destruction of buds and flowers, production of embryoless seeds and reduction of seed yield.

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## DISCUSSION

M. A. WATSON (MRS.). Do the stylets penetrate right to the embryo or is there a transfer of a toxin to it?

F. FLEMION (MISS). They feed directly on the embryo.

T. E. MITTLER. Did you observe relative movement of the maxillary stylets during penetration?

F. FLEMION (MISS). Yes, as shown in histological material as well as direct observation through the microscope.

T. E. MITTLER. As the saliva cannot aid chemically in such rapid penetration of the cell walls, what other function can you suggest for the saliva?

F. FLEMION (MISS). This is of considerable interest. It might be part of the suction-pumping mechanism.

T. E. MITTLER. I am surprised that saliva was ejected from the stylets (during collection for radioactivity assay) while the stylets were not inserted in the host plant.

J. S. KENNEDY. In how many planes can the stylets be moved by the feeding insect?

F. FLEMION (MISS). From our observations it appears that they move in many directions.

J. S. KENNEDY. Are the cells which are emptied by the feeding insects then filled with saliva?

F. FLEMION (MISS). They deposit saliva very shortly before withdrawing from the tissue.

R. H. PAINTER. Did you find any evidence of stylet sheath material in the stained slides.

F. FLEMION (MISS). None.

J. C. ARRAND. Some recent work has shown that fairly large quantities of pectinase are secreted by some species of aphids. If *Lygus* bugs secrete pectinase when feeding, that might be the cause of some of the damage.



# Experiments on the Integration of Biological and Chemical Control of Insect Pests

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## ABSTRACT

*Approaching the integration of biological and chemical control from the side of chemical control and accepting that it will for some time remain in the domain of supervised entomology, we have so far not met any unsurmountable obstacles of an entomological or economic nature.*

*For certain pests, viz. those of man and animals, control by the purely chemical method is the only answer. But for crop pests, where natural enemies occur or have been artificially introduced, the integration of chemical control with this natural defence system of the plant association might be more economic.*

*It is recognised that this approach is still very much in its beginning, but it provides a stimulus to ecological, physiological, and toxicological research, and may perhaps lead to a modified way of control of phytophagous pests.*

High agricultural productivity often depends on large yield increases gained by the chemical control of pests. Because of the many references to undesirable sequels to pesticide applications, investigations have been undertaken to determine whether chemical control as practised to-day is a temporary palliative, which may raise yields in the short run but could, over a period of years, end by saddling agriculture with pests that are impossible to wipe out. The increasing number of strains of resistant pests and the growing reports of resurgences of pests after chemical treatments are, in the present economics of world agriculture, not yet important, but they have been regarded as potential storm signals and have stimulated the search for an adaptation of the chemical control method to avoid the evolution of resistant strains and other undesirable sequels. Because many of these are traceable to unfavourable effects of the pesticides on the natural enemies of phytophagous insects, Pickett and collaborators in Canada (Pickett, 1949; Pickett and Patterson, 1953; Lord, 1956), Clancy (1955, 1956), and Bartlett (1955) in the U.S.A., and Massee (1954) and Ripper (1949, 1950, 1951) in England have attempted to combine the biological and chemical control of pests.

Such work was based on the concept that plants are biological systems. When plants become infested by phytophagous insects and mites, these in turn attract a population of their natural enemies, thus forming a defence mechanism for the crop. Like the defence system against foreign bodies in vertebrates, this defence mechanism of natural enemies can only cope with moderate infestations of phytophagous pests (Collyer and Kirby, 1955). Once the pest infestation has reached a high level, only pesticide applications have so far succeeded in correcting it, but experience has shown that pesticides prove more successful in the long run if they are selected so as to eliminate or reduce the offending pest population only, and do not destroy the defence mechanism (Ripper, 1956).

Where the natural enemies are destroyed, survivors of the pest population multiply more quickly than on untreated plants because the natural enemies no longer restrict the growth of the pest population which resurges to outbreak dimensions very quickly after spraying. The analogy between this phenomenon and the enhancing of bacterial infections of mammals after damage to the reticuloendothelial macrophages by cortisone comes to mind.

Where the pesticide kills the pest against which it is aimed, but along with it the natural enemies of other phytophagous species of minor importance, a removal of these natural enemies by the pesticide leads to an outbreak of that phytophagous species; outbreaks of "insecticide-made" pests show some resemblance to the repercussion of the microbiological flora of the digestive tract of mammals to massive doses of antibiotics which often lead to the domination of the flora by previously suppressed and often undesirable microorganisms.

We have shown six years ago (Ripper *et al.*, 1950) that selective insecticides, i.e. pesticides which kill the phytophagous species but do not affect its natural enemies, do

<sup>1</sup> Present address: Docking, King's Lynn, Norfolk.

not cause resurgences of the species against which they have been directed, or other phytophagous insects present in the ecosystem.

As resurgences are caused by a rapid multiplication of survivors of the insecticide treatment after removal of the natural enemies, they do not occur if the mortality of the phytophagous species is 100 per cent. Their place is, however, often taken by reinfestations which occur rapidly after complete mortality effected by a pesticide, if the migrants of the pest invade crops completely freed of phytophagous species and their natural enemies. Re-infestations lead to outbreak conditions faster if the biotic resistance of the treated ecosystem has been decreased.

Boyce (1936), Nicholson (1947), and Wigglesworth (1945) have drawn attention to the fact that a reduction of phytophagous species to a low population density by the application of pesticides can produce an increase of the biotic potential of the pest as a consequence of the simultaneous reduction of biological control factors, whether due directly through the insecticide killing the natural enemies or indirectly by their being starved out. This latter phenomenon can also occur with selective insecticides (Ripper, 1944), and poses a question as to whether there are better ways of using selective insecticides.

In an ecosystem where natural enemies are effective, a pest outbreak occurs when reduction of the pest by the natural enemies becomes inadequate. If selective insecticides could be used to offset only the effect of the factors which have upset the balance of population without reducing the pest population so that the natural enemies eliminate them completely and then disappear themselves, a new equilibrium between the pest and the low density natural enemies would establish at a lower population level.

The level to which populations of the pest must be reduced is that which does not damage the crop; were it possible to achieve that, and leave the pest population sufficiently high to maintain its low density dependent natural enemies present in the ecosystem, the *first target of integration of biological and chemical control* would be effected.

The most dangerous sequel to pesticide application, the development of resistance, is not possible when the survivors of the pesticide treatment are mopped up by the natural enemies after the use of a selective insecticide. It is questionable whether an integration as just described is effective in preventing the development of resistance. Where the development of resistance is to be feared, the method of integration of biological and chemical control may therefore have to be modified; at suitable time intervals the dosage of the selective insecticide might be so increased that the natural enemies can, after a period (for instance towards the end of the vegetation period), practically eliminate the surviving individuals of the pest in the treated ecosystem (*second target of integration*).

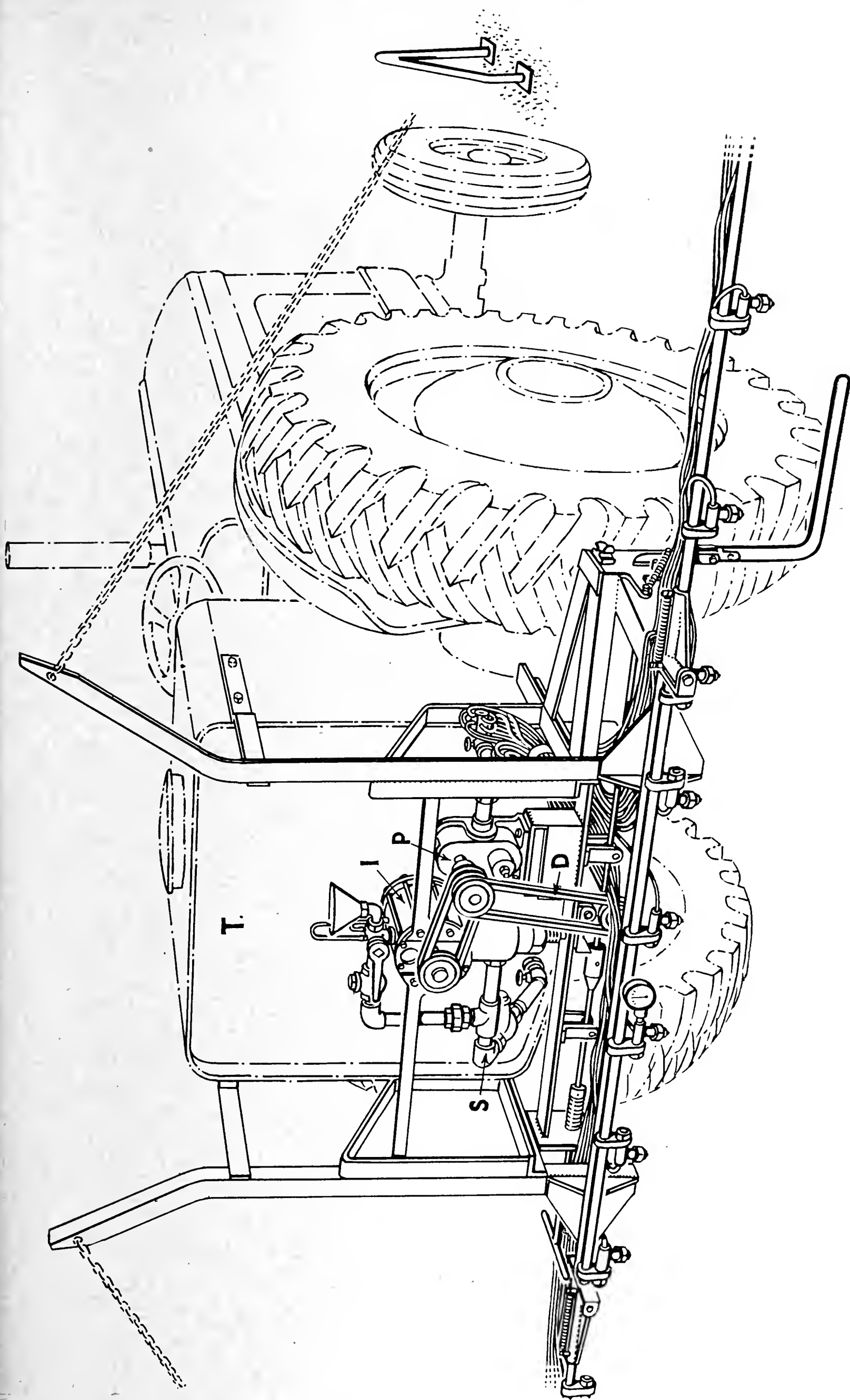
## METHODS

To experimentally vary the balance of arthropod populations present in the ecosystem is possible by many methods, but, to make the rapid progress which the present situation requires, a technique had to be developed which reduced labour to a minimum.

Huffaker and Kennett (1953) have successfully used manual removal of predators to alter the balance, but as the chemical method of control is pertinent to this investigation, it was used exclusively. The data required were as follows: (a) the mortality of the pest after pesticide treatment, (b) the mortality of the pests' natural enemies, and (c) the lowest pest population density which still holds the natural enemies and the yield from crops whose pest population density had been recorded.

It was found that all these data could be obtained on suitably-replicated plots using a logarithmic sprayer. For this purpose a variable dosage sprayer, the Chesterford Logarithmic Sprayer, originally designed for weed control experiments by Pfeiffer, Brunskill and Hartley (1955, 1956) was suitably modified for research in experimental population dynamics (Fig. 1). As equivalent increases of biological response are often produced by equivalent relative increases of concentrations, the sprayer had been designed so that the

Fig. 1. Chesterford Logarithmic Sprayer adapted for entomological work and attached to a tractor-mounted low volume sprayer. (I) dilution vessel, (P) gear pump, (S) supply tube from tank of sprayer (T), (D) drive from power take-off.



spectrum of dosage should be on a logarithmic scale and the dosage is halved for every additional standard distance of travel of the sprayer. This is obtained by dilution during the spray run. The liquid is pumped to the spray nozzles from an intermediate constant volume dilution vessel (I) which is otherwise closed during the spray run, except for a tube leading from a supply of diluting liquid (S). This dilution vessel is equipped with an efficient agitator (A) and is initially completely filled with the chemical at a concentration rather higher than the maximum expected to be of interest. The delivery from the dilution vessel is started by an iron stake (ST) which activates a spring-loaded valve (V). This tripmarker is placed on the soil at the starting line of the plot before the treatment commences (Fig. 2). The pesticide is pumped from the intermediate vessel to the nozzles which all have equal output at a given pressure through separate narrow tubes of equal length from two manifolds fitted close to the pump, so that the same concentration reaches all nozzles simultaneously.

As spraying proceeds, liquid is removed from the intermediate vessel and is automatically replaced by an equal volume of diluent. The mathematical results have been fully discussed by Hartley *et al.* (1956). The concentration decreases exponentially with the volume delivered and as one arranges the machine to spray at a constant volume per yard run, the concentration delivered on to the ground decreases as an exponential function of the distance travelled.

When spraying is carried out from a tractor moving at constant speed, the volume sprayed out is proportional both to time and to distance. The proportionality to distance is of primary importance in this type of experiment, since the concentration is then an exponential function of distance along the plot; in other words, the distance is a logarithmic measure of the concentration. A gear pump directly geared to the tractor was used to ensure that its output was approximately proportional to speed. Care was essential to obtain the correct revolution on the tractor, and a thorough overhaul and tightening of the governor linkages was necessary. A Tachometer was required. While the distance over which the concentration of the spray chemical is halved in herbicidal experiments varies between 5.5 yards (Hartley *et al.*, 1956) and one yard (Fryer, 1956), the entomologist requires distances to halve the concentrations between 15 and 30 yards for aphids, whiteflies and pests of similar mobility because for studies in population dynamics a much slower decrement of the concentrations is required in order to have larger areas of similar concentrations. This was achieved by using larger intermediate vessels exchangeable with each other which had a volume of 1.5, 4.5 and 14 litres.

To obtain ecological selectivity, directional spraying as practised in weed control was sometimes necessary. For this purpose the nozzles on the sprayer were made detachable and could be clamped on downarms, so that they sprayed a band of soil and only wetted the stem of the plants and not the leaves.

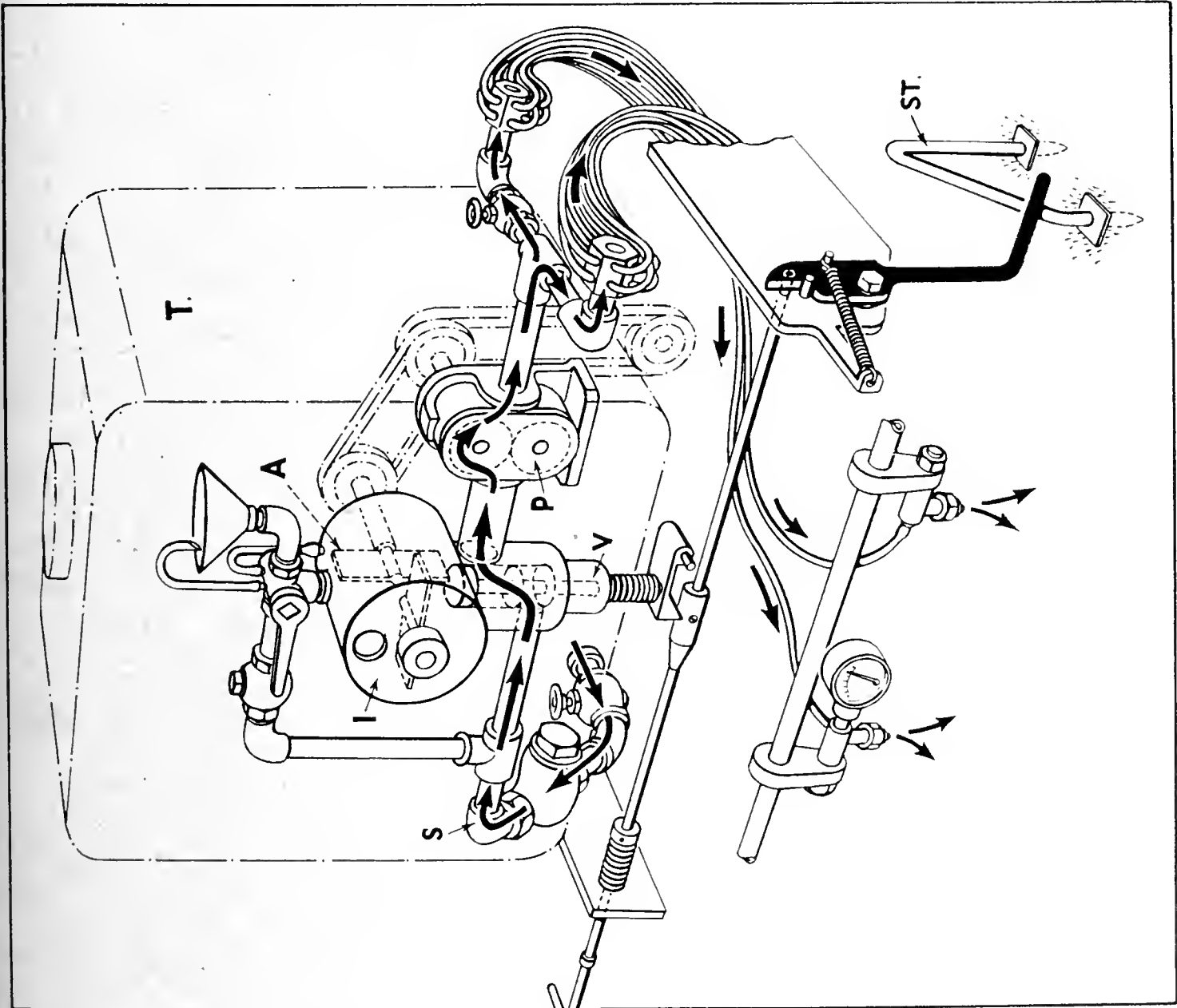
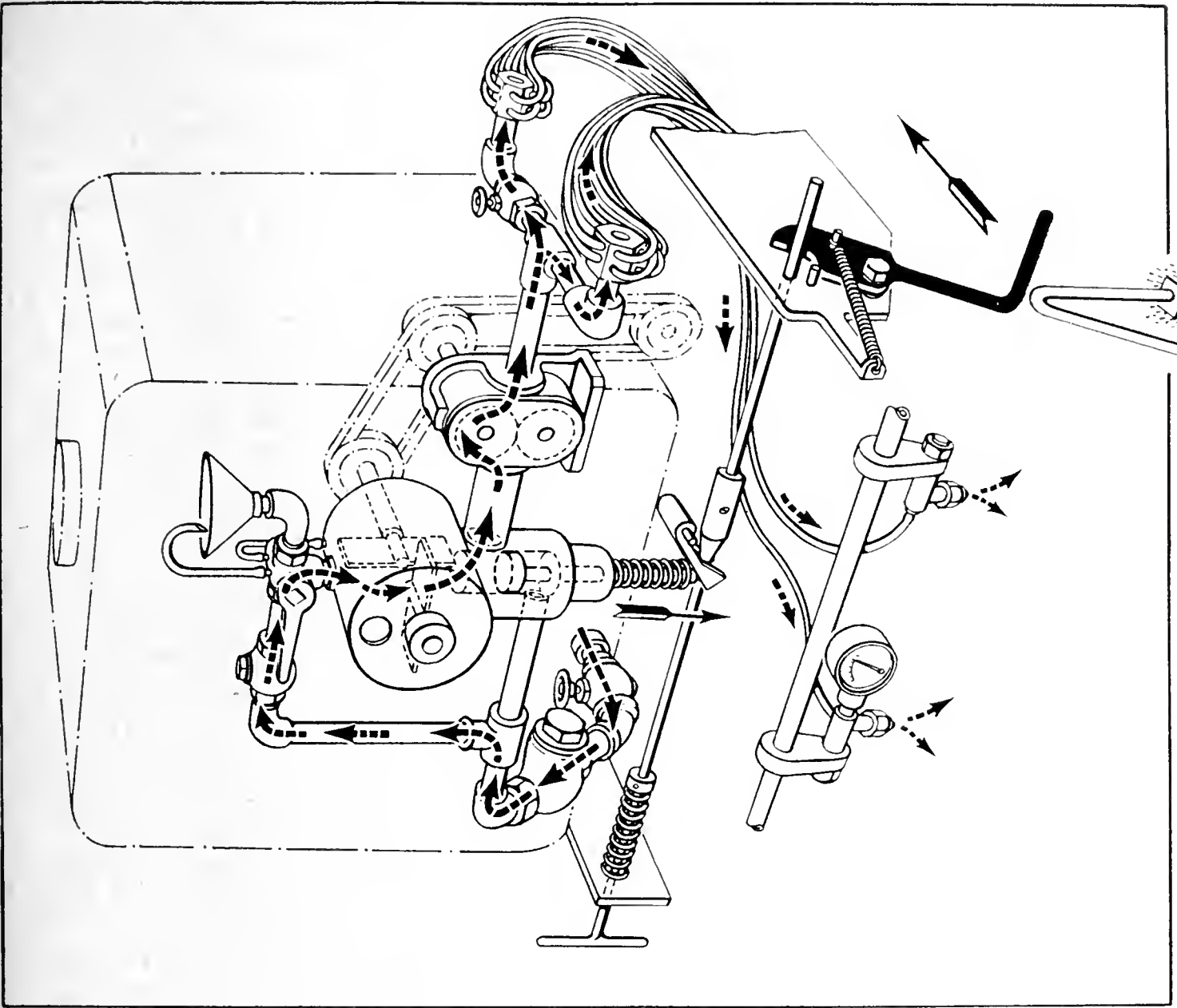
As the entomologist carries out his evaluation by walking along the plot, making counts where the phenomenon under investigation seems to have occurred, it is necessary to erect distance indicators on poles along one or both sides of the plots showing the distance from the starting point (Fig. 3). Given the distance (X), the relevant dosage can be read off a Nomogram (Fig. 4) as fraction of initial peak dosage ( $D/D_0$ ). Replications of logarithmic spray plot were laid down either in sequence or side by side; with the decrement taking place in the same or opposite direction. In the latter case the distance indicators were in two colours to easily distinguish the indicator belonging to each direction of the decrement. The aphid counts were carried out according to the method described by Strickland (1952), that is, selecting three leaves per plant at three levels of the plant, and sampling ten plants per observation point.

To obtain yield data related to the population density of the pest, the relevant section of the plot was marked off as a strip and harvested. There were four replications of each logarithmic plot, but the statistics of this method is still under study.

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Fig. 2. Flow diagram for Logarithmic Sprayer. (I) dilution vessel, (A) agitator, (S) supply tube, (P) pump, (ST) stake which activates spring-loaded valve (V) and thereby changes the flow of the liquid as in A (solid lines) to that of B (dotted lines). For illustrative purposes the pump drive has been shown behind the pump.







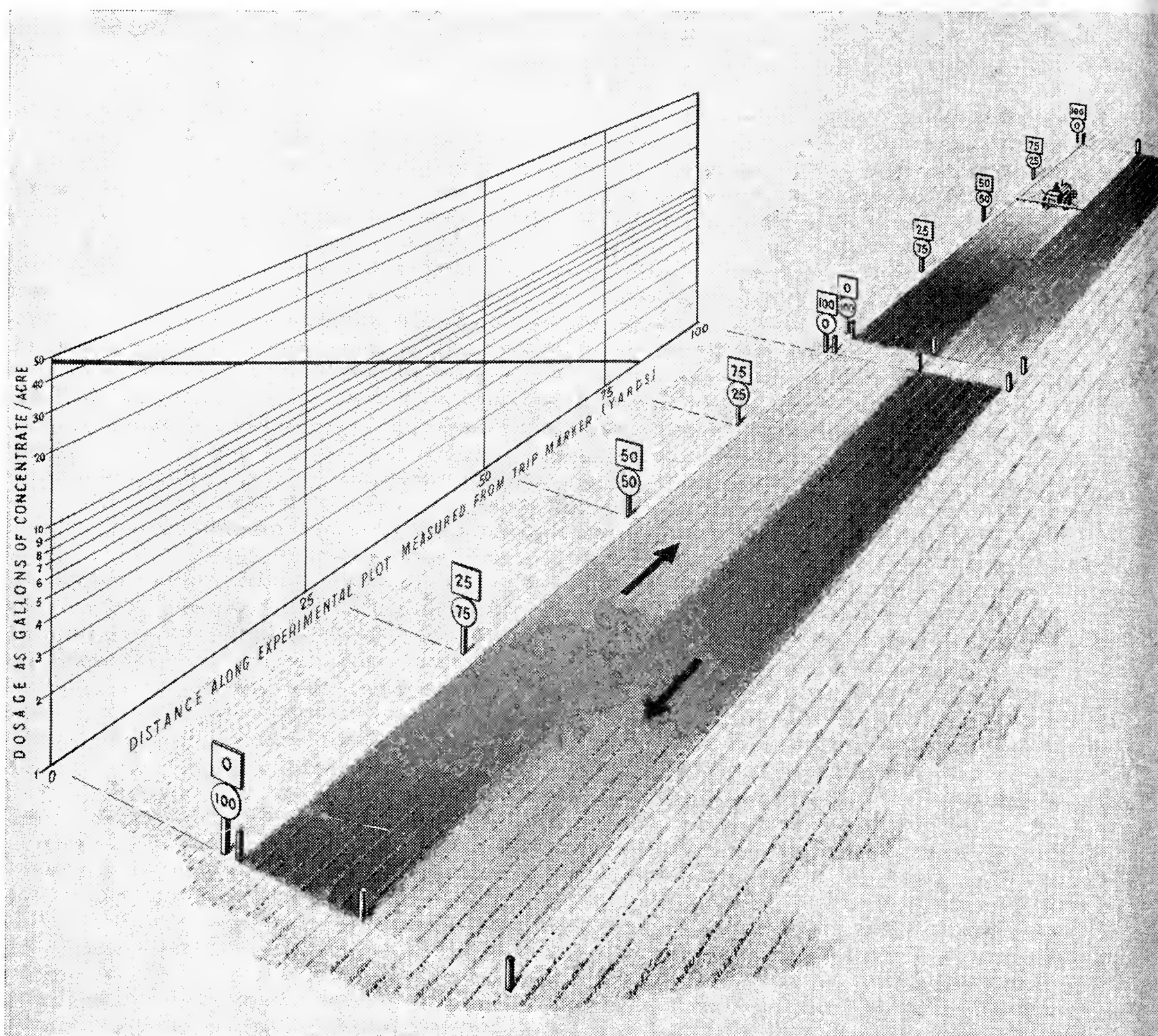


Fig. 3. Schematic drawing of four plots sprayed with the Logarithmic Sprayer. The higher concentration of the insecticide is indicated by deeper shades. Beside the plots are the distance indicators which give the distance in yards from the starting line when travelling in either direction. The calibration curve of the logarithmic sprayer with the 4.5 litre dilution tank as used for population studies is shown on the left hand side of the plots for a sprayer moving towards the background.

The data thus obtained for the population densities of pests and natural enemies can be represented by a tri-dimensional system plotting population density against pesticide concentrations and time, or a series of curves for selected concentrations of pesticide in a system plotting population density against time.

### EXPERIMENTAL RESULTS

Experiments were designed to test the practicability of the hypothesis of integration on simple pest complexes. From previous experience very different reactions of the pest population to the treatment were expected with non-selective and with selective insecticides, depending whether the pest is beset by a low density-dependent natural enemy or not. In the first case, where effective low density natural enemies are at work, a resurgence is likely with a non-selective insecticide, while with a selective, no such sequel is expected. In the second case, where no effective low density dependent natural enemies are present, the reaction to either type of the insecticide should be similar. If this was established, a comparison of the effect on the pest population after selective and non-selective insecticide treatment can be used as a method to determine whether effective biological control factors are at work.

The results of experiments with cabbage infested by *Brevicoryne brassicae* which was parasited by *Diaretus rapae* give us an example of the first case.

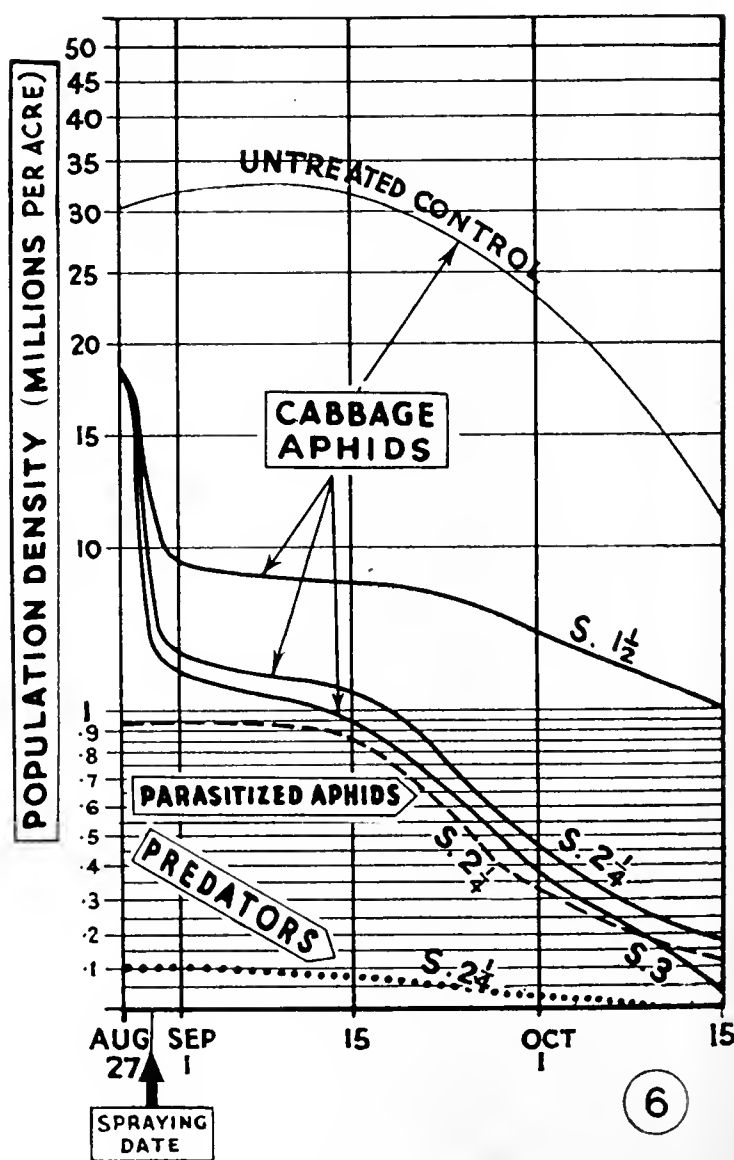
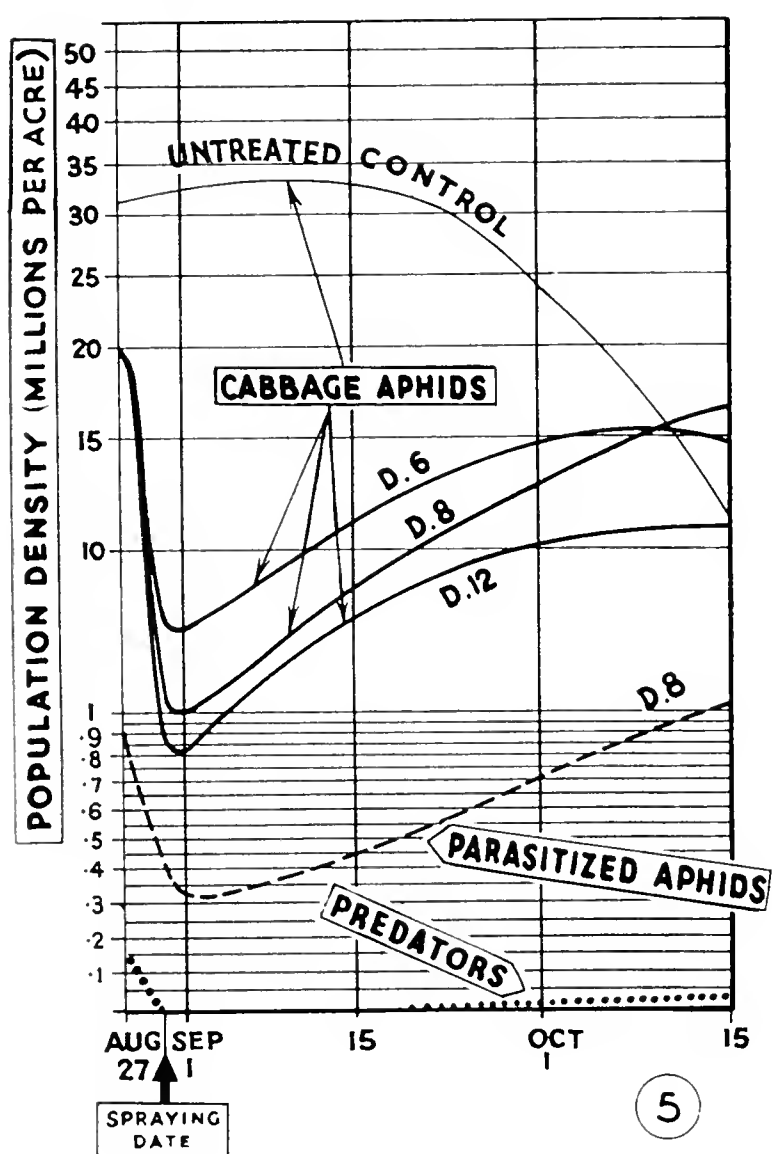
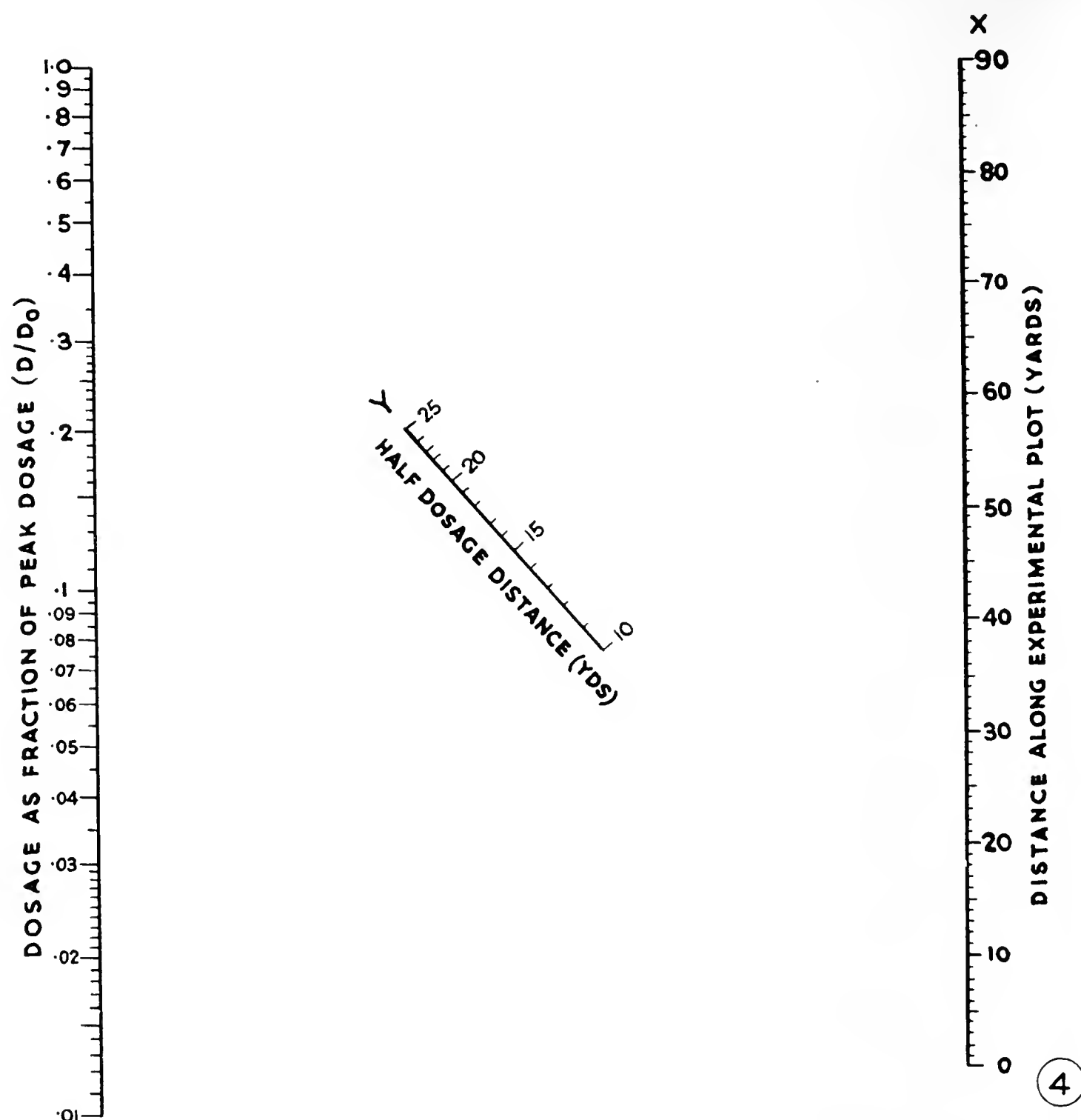
The effect on aphid populations of three systemic insecticides was compared; we selected one selective systemic insecticide (schradan) and two unselective ones (demeton and methyl-demeton). For each systemic insecticide, records of the population of the pest and its natural enemies were taken at three levels of concentration, selecting the highest concentration commercially recommended, and two lower levels. These were as follows: schradan, 3 pints,  $2\frac{1}{4}$  pints and  $1\frac{1}{2}$  pints of a commercial formulation containing 66% octamethyl-paraphosphoramidate and other amides (Pestox 3) ® per acre; demeton, 12 ozs., 8 ozs. and 6 ozs. of a commercial formulation (Systox ®) per acre; and the same quantities of methyl-demeton, (Metasystox ®). In all cases, the application was carried out after the infestation had built up to 20 million aphids per acre, and there were 950,000 parasitized aphids per acre on the foliage; aphids were counted as parasitized when they had become immobile, and no distinction between hibernating and hatching *Diaretus rapae* was made. The predators present were Syrphid larvae, *Catabomba* sp. The plots were kept under observation until the end of the season; on the control plots the infestation increased until the middle of September, but from that time the aphid population decreased, as predators became more abundant and the parasitism slowly mounted. Towards the end of the year the parasites had virtually wiped out the infestation.

On the plots treated with the non-selective demeton (Fig. 5) and methyl-demeton a good mortality of aphids was effected, resulting in a considerable initial drop of the aphid population, but thereafter the aphid infestation resurged to population densities in the neighbourhood of 15 million per acre; at the highest level of methyl-demeton, this resurgence was less pronounced. Associated with the drop in the aphid population there was a comparable drop in the population parasitized aphids; demeton wiped the predators out completely, but when the resurgence of aphids was well under way, new immigrant predators re-established themselves. With methyl-demeton the drop in predator population was a little less pronounced. This confirms other experiments carried out concurrently which showed that methyl-demeton is slightly less toxic to Syrphid larvae than is demeton.

When the aphid population data after the schradan treatment are studied, a different effect on the insect population becomes obvious (Fig. 6); at an application rate of 3 and  $2\frac{1}{4}$  pints of schradan the aphid population continued to decrease during the period after treatment, and the crop was, for practical purposes, clean on October 15th. The population of the pest had been kept sufficiently high to support an effective parasite population which coped with further newly arrived migrants of the cabbage aphid, thus the crop was kept commercially clean for the remainder of the season.

The effect of the selective as compared to the non-selective insecticide on the balance of the insect population becomes even clearer if the result of these experiments are plotted as the ratio of number of parasitized aphids against the population of cabbage aphids (Fig. 7); on the untreated plots from the middle of September, the ratio of parasites to pests increases as the natural enemies slowly effected a decline of the aphid population. This ratio is much improved by the schradan treatments, which as mentioned before, are not toxic to the natural enemies of the cabbage aphid. As the high density dependent natural enemies (Syrphids and Cecidomyids) leave the crop, the steep rate of the ratio parasite pest flattens out and leads to a less steep slope when the low density natural enemies are at work on their own. Methyl-demeton and demeton treatments very much reduced the ratio of parasitized aphids to pests. Similar difference in behaviour of schradan and demeton against another aphid has been reported by Michelbacher and colleagues (1955a, 1955b) in California.

The experiment shows that in the presence of low density dependent natural enemies a selective systemic can be used to alter the balance between aphids and their natural enemies in such a way as to maintain the pest populations after treatment at a reduced level which will retain the low density dependent natural enemies on the crop. This is then an example of the first target of integration of biological and chemical control. With a slightly higher dosage of the selective systemic insecticide than  $2\frac{1}{2}$  pints of Pestox 3 ® the balance between aphid and the parasite can be so altered that the latter achieves a complete eradication of the surviving aphids at the end of the growing season and thus provides an example of the second target of integration.





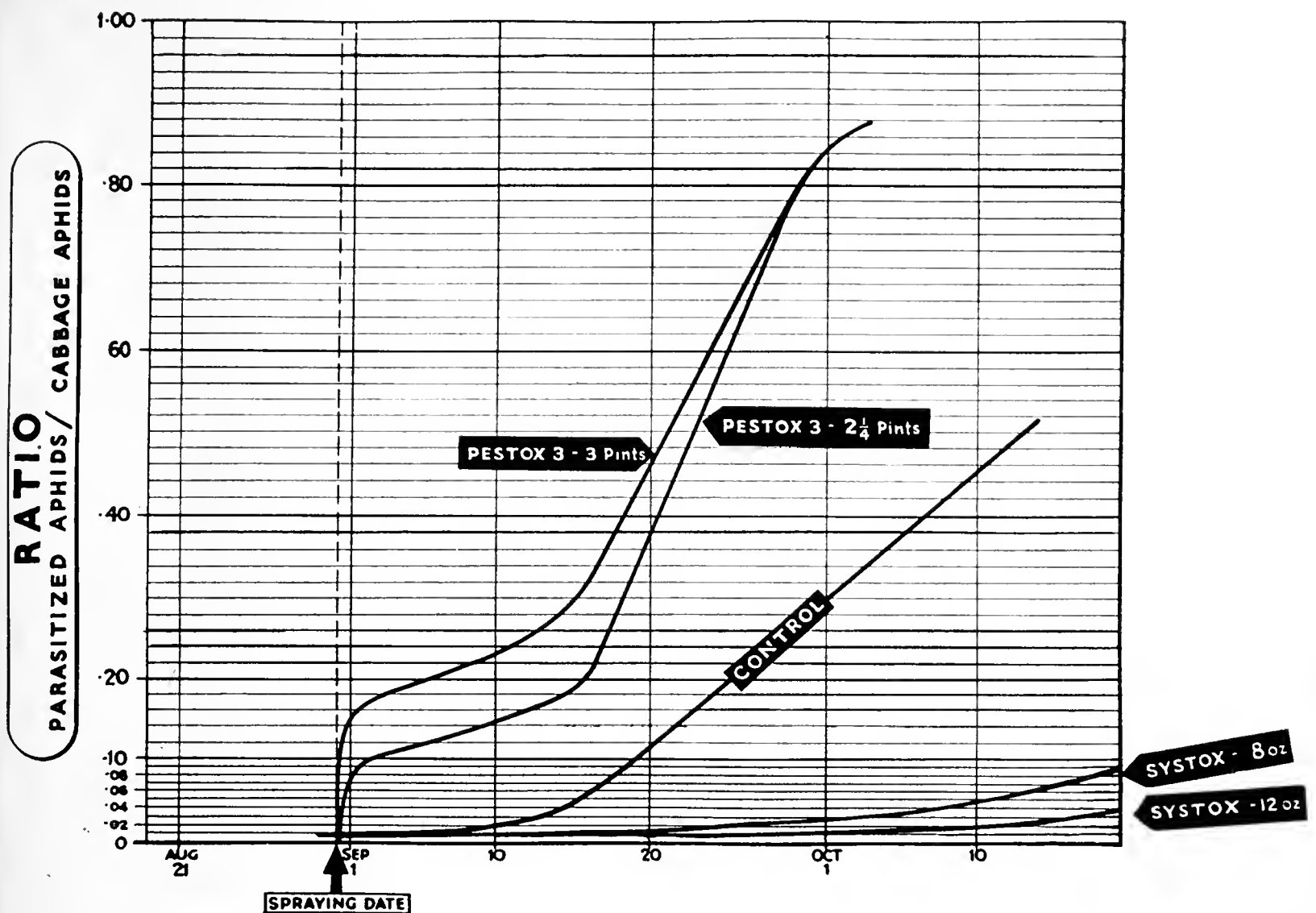


Fig. 7. Ratio of the population of parasitized aphids and the cabbage aphid plotted for the experiments illustrated by Figures 5 and 6. Note contrast in the effect of a selective (Pestox 3 ®) and an unselective (Systox ®) insecticide on the balance between a pest and its natural enemies.

To corroborate these findings we carried out similar experiments against *Aphis gossypii* on cotton on the Blue Nile in the Sudan, where the aphid was preyed upon by Coccinellid larvae, but no parasite was found. Again schradan served as a selective systemic and was compared with five non-selective systemics, viz. demeton, methyl-demeton, dithio-demeton, FAC 20 (containing N-monoisopropylamide of 0,0 diethyldithiophosphoryl acetic) and Roger ®, the methyl homologue of FAC 20. When the aphid populations were plotted, no great differences between selective and non-selective insecticides were apparent and the reinfestations developed along similar lines.

Although the cotton plants treated with schradan remained longest toxic to the cotton aphid because of its longer persistence in the plant, the effect of the various pesticides on the populations of the aphid indicated that no low density natural enemies were at work, and none were found by careful examination of the untreated crops. The Coccinellid predator, *Scymnus marginalis* Rossi, became plentiful on unsprayed and sprayed crops when the population density reached a certain level. On sprayed plants the Coccinellid disappeared when the population density fell under 500 aphids per 30 leaves, even when the cotton was sprayed with schradan which does not kill the ladybird larvae either by ingestion or by contact.

As a next step an integration experiment involving two different pests was conducted. The cabbage aphid and the diamond-back moth occur together, and cause considerable

Fig. 4. Nomogram expressing the spray dosage of the logarithmic sprayer as a fraction of the initial dosage placed in the dilution vessel ( $D/D_0$ ).

Fig. 5. Effect of demeton on the cabbage aphid on brassicas when used at various rates ( $D_{12}$ ,  $D_8$ , and  $D_6$ , = 12, 8, and 6 ozs. of Systox ® per acre respectively). The population of the parasitized aphid and the syrphid predators are plotted for the medium level ( $D_8$ ) of the demeton concentration only. Note the resurgence of the aphid population.

Fig. 6. Effect of schradan on the cabbage aphid on brassicas when used at three rates ( $S_3$ ,  $S_{2\frac{1}{4}}$  and  $S_{1\frac{1}{2}}$  = 3,  $2\frac{1}{4}$  and  $1\frac{1}{2}$  pints of Pestox 3 ® per acre respectively). The selective insecticide has not reduced the natural enemies and no resurgences of the aphids are observed. The population of the parasitized aphid and the syrphid predators are plotted for the medium level ( $S_{2\frac{1}{4}}$ ) of the Schradan concentration only.

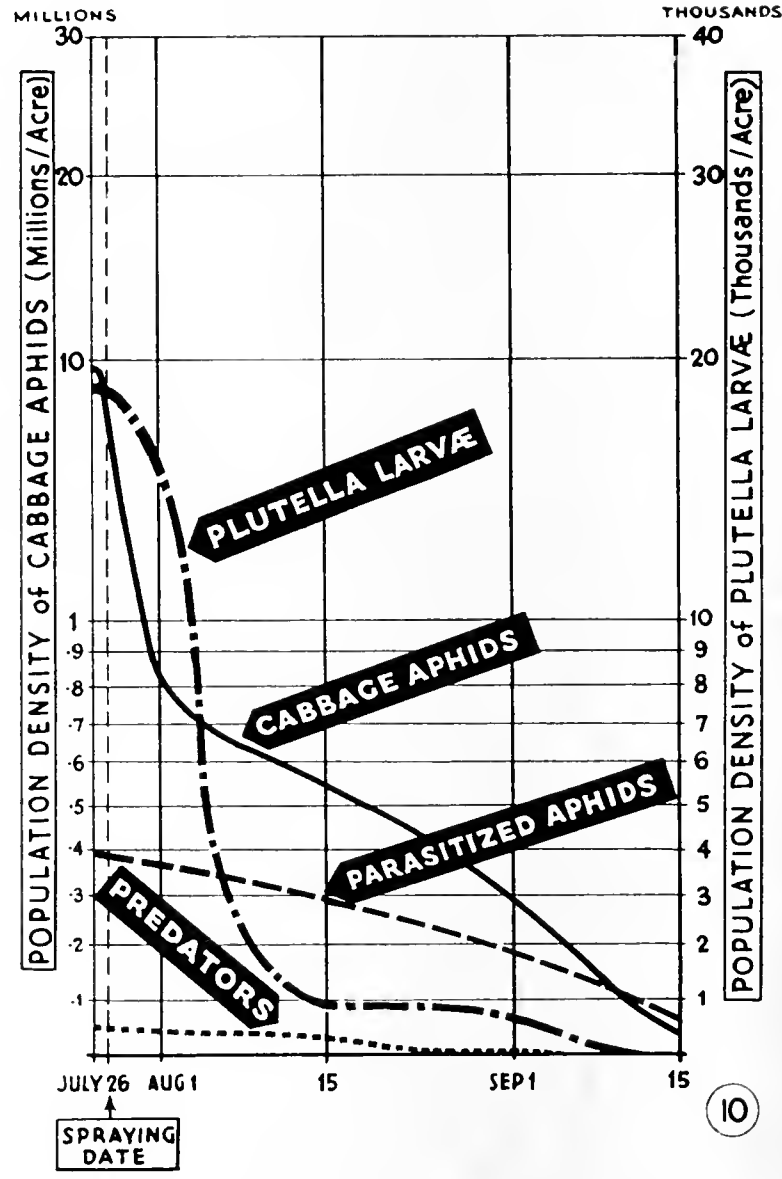
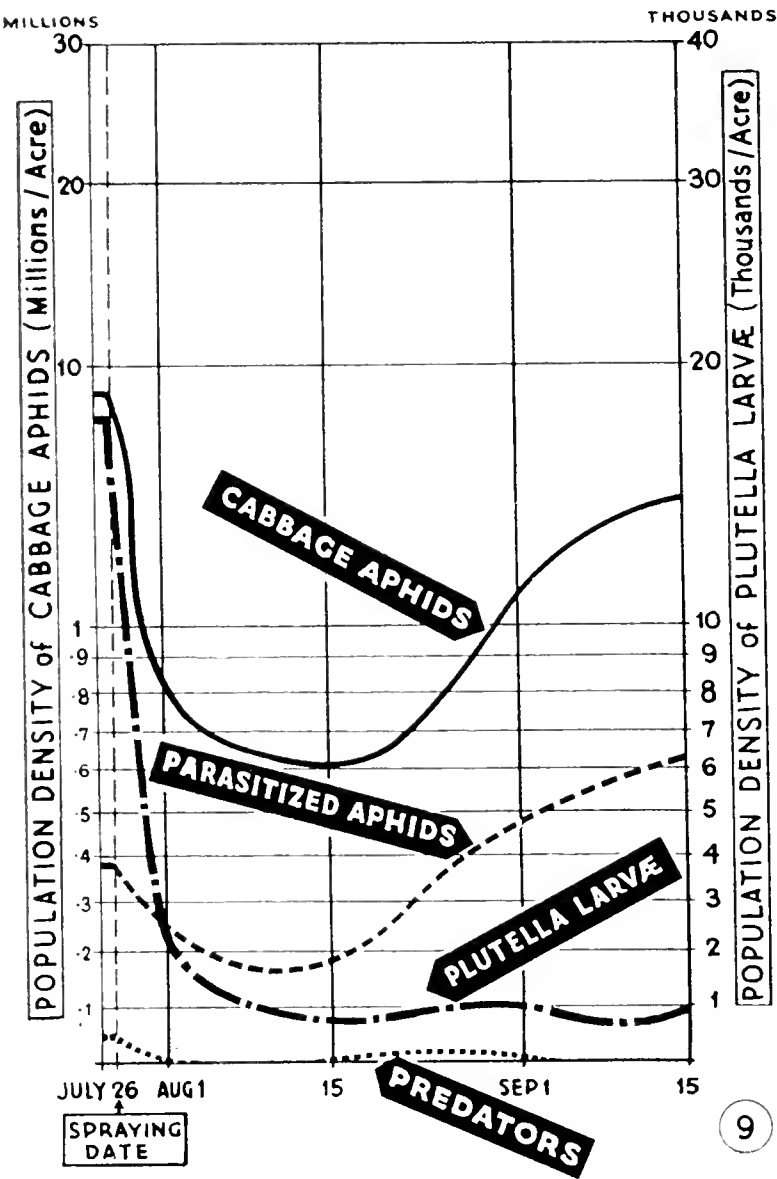
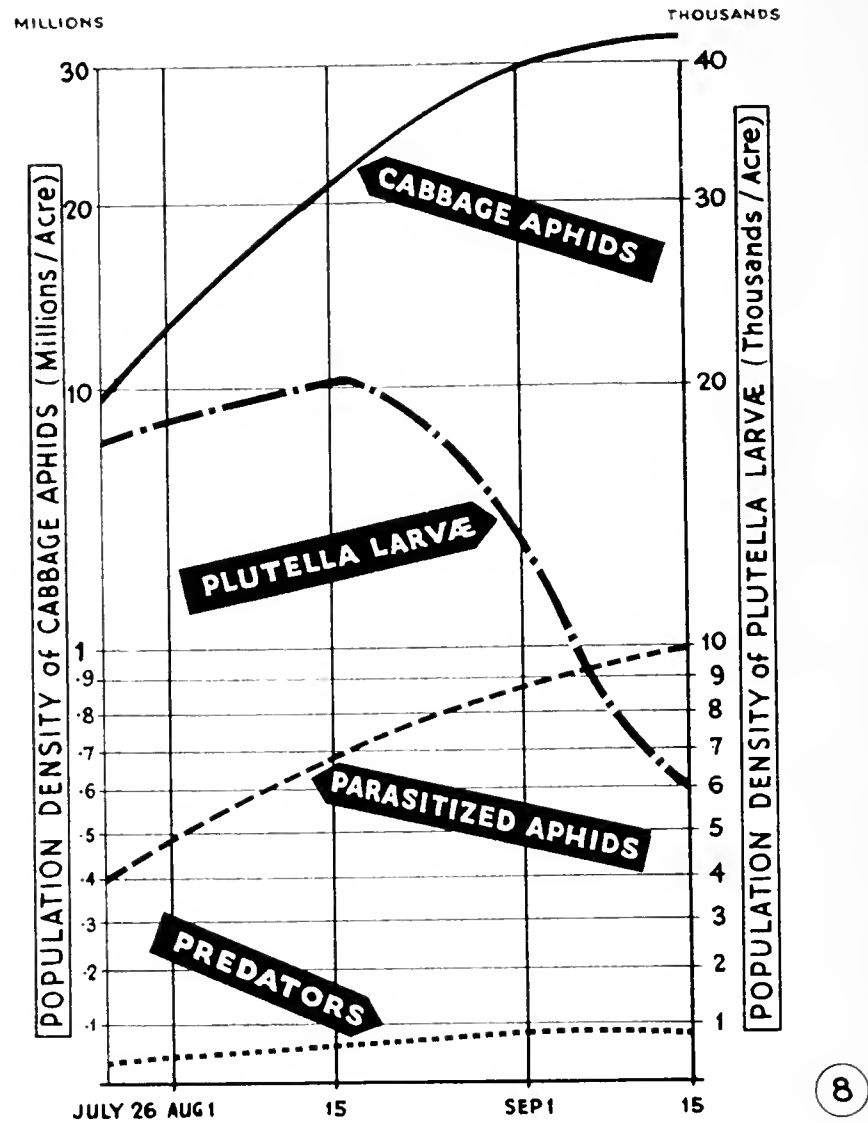


Fig. 8. Populations of the cabbage aphid, *Brevicoryne brassicae*, of its parasite *Diaretus rapae*, and of the larvae of *Plutella maculipennis* on an untreated brassica crop.

Fig. 9. The effect of schradan and a suspension of DDT on the population of the same insects as in Fig. 8. Note the resurgence of the cabbage aphid and the reduction of its natural enemies after treatment.

Fig. 10. Effect of a treatment with schradan and zein-coated DDT. This has resulted in similar control of the *Plutella* larvae as in Fig. 9 without deleterious effect on the parasites of the cabbage aphid.



damage to cabbage crops (Fig. 8). If the aphid is controlled by a selective aphicide (3 pts. of 60% schradan preparation), the application of a DDT suspension (1 lb. DDT per acre) for control of the diamond-back moth upsets the natural enemies of the cabbage aphid. A thorough application of DDT destroys not only the *Plutella* larvae, but severely reduces parasitism by the aphid *Diaratus rapae*, and kills most of the predators; a few weeks later a resurgence of the cabbage aphid results, which is similar to that caused by a non-selective aphicide (Fig. 9). An experiment was therefore undertaken to integrate the biological control of the aphid with pesticide applications against the aphid and the *Plutella* larvae. As selective insecticides we used schradan for the aphid and zein-coated DDT against the *Plutella* larvae. From previous experiments the target population density of the cabbage aphid was known, and it had been established that 2¼ pints of schradan would produce a pseudo-equilibrium between *Diaratus rapae* and the cabbage aphid at a lower economically unimportant population level.

To obtain a stomach poison effect on the *Plutella* larvae without affecting *Diaratus* we used a DDT suspension coated with zein, which had previously been shown to have a low contact toxicity but was still a good stomach poison (Ripper *et al.*, 1948). The zein coating was so applied that a zein: DDT ratio of 1:1.25 (formulation D<sub>2</sub>) and of 1:4 (D<sub>3</sub>) was obtained. In the laboratory with *Drosophila melanogaster* and *Tribolium confusum* it was shown that the contact toxicity of the D<sub>2</sub> preparation was very much less than that of the standard DDT suspension. For this purpose 4-inch diameter glass plates were treated with an aqueous suspension of the formulations. Concentration of the material was adjusted so that 1 cc. of solution painted over the glass plates evaporated to give a deposit of DDT equivalent to 10, 5, or 1 mg./square foot. Results are the average of three replications of approximately 40 flies (Tables I and II). The insects were confined to the plates by glass rings for one hour and then incubated at 25°C for 23 hours before counting.

The stomach poison effect of DDT was maintained against caterpillars of *Pieris brassicae* despite the zein coating of formulations D<sub>2</sub> and D<sub>3</sub>, when the DDT formulations were sprayed on cabbage leaves (Tables III and IV).

Formulation D<sub>2</sub> controlled the *Plutella* larvae in the field at a rate of 1 lb. of DDT per acre, and did not affect the natural enemies of the aphid or the diamond-back moth. The kill of the diamond-back moth larvae was as high as in the standard DDT suspension, and counts of the population of the two pests and the natural enemies of the aphid showed that a good integration of biological and chemical control had been obtained (Fig. 10).

These experiments are in agreement with the hypothesis of integration put forward above. The question arises whether a successful integration can also be achieved in still

TABLE I. *Drosophila melanogaster* on Glass Surfaces.

Material	Deposit mgs. DDT/ft <sup>2</sup>	% mortality when exposed at time intervals after treatment of surface						
		2 hours	1 day	5 days	7 days	10 days	14 days	21 days
D <sub>1</sub>	10	100	100	100	100	100	56	23
Standard DDT suspension	5	100	100	74	80	62	22	5
	1	41	10	10	10	27	0	—
D <sub>2</sub>	10	94	33	40	40	20	8	6
Zein: DDT 1:1.25	5	58	21	0	—	—	—	—
	1	11	0	0	—	—	—	—
D <sub>3</sub>	10	100	100	100	100	90	66	11
Zein: DDT = 14:	5	44	50	58	40	18	28	—
	1	13	0	0	—	—	—	—

TABLE II. *Tribolium confusum* on Glass Surfaces. Plates Treated in the same Way as for *Drosophila*, but the Insects were Confined on Surfaces for 24 Hours and Incubated at 25°C for 1 Week before Estimating Mortality.

Material	Deposit mgs. DDT/ft. <sup>2</sup>	% mortality when exposed at time intervals after treatment of surface					
		1 day	3 days	5 days	7 days	14 days	21 days
D <sub>1</sub>	10	97	100	80	22	28	5
	5	84	38	48	5	8	2
	1	43	7	2	3	—	—
D <sub>2</sub>	10	88	18	48	12	0	2
	5	30	7	12	8	2	—
	1	5	7	2	0	—	—
D <sub>3</sub>	10	100	55	65	42	32	5
	5	82	68	5	3	2	0
	1	17	2	5	3	—	—

TABLE III. *Pieris brassicae* (2nd instar larvae) on Cabbage Leaves.

Material	Deposit mgs/ft <sup>2</sup>	% Mortality: Age of deposit							
		2 hours		3 days		5 days		7 days	
		24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
D <sub>1</sub>	10	100	—	100	—	100	—	100	—
	5	100	—	100	—	100	—	100	—
	1	100	—	30	57	29	72	52	*
D <sub>2</sub>	10	100	—	100	—	100	—	100	—
	5	100	—	67	95	85	95	80	*
	1	71	100	79	90	80	95	31	78
D <sub>3</sub>	10	100	—	91	91	100	—	100	—
	5	100	—	81	94	82	95	100	*
	1	100	—	40	75	32	90.	33	66

\*Insects escaped from cages overnight.

more complex pest situations. From the experiments so far carried out on the simpler pest complexes, the following requirements for a successful integration of biological and chemical control can be deduced.

PREREQUISITES FOR INTEGRATION

Integration of biological and chemical control is possible if, (a) a suitable low density natural enemy is available, (b) a selective insecticide exists which controls the pest but not the low density dependent natural enemies, and (c) the maximum population density of the pest which does not produce economically significant damage to the crop is not

TABLE IV. *Pieris brassicae* Larvae (final instar) on Cabbage Discs (9.6 cm<sup>2</sup>). Larvae Allowed to Feed on Treated Disc for 24 Hours After which it was Removed and Fresh Untreated Discs Fed to Larvae (2 Repetitions).

Material	Deposit mgs/ft <sup>2</sup>	% mortality after 5 days	% of treated leaf disc consumed
D <sub>1</sub>	10	33	8.9
	5	17	63.0
	1	17	100
D <sub>2</sub>	10	50	23.0
	5	20	91.5
	1	0	100
D <sub>3</sub>	10	50	14.0
	5	17	46.0
	1	0	100

lower than the population density of the pest needed to retain the low density natural enemy.

#### SUITABLE BIOLOGICAL CONTROL FACTORS

Populations of pests on crops are only economically acceptable at a level which does not cause damage to the crop. Hence low density dependent natural enemies are, in the first place, of interest for the integration of biological and chemical control. A good deal of information on the subject exists, but it is not readily accessible, and it would be useful for our purpose if, in the relevant papers, such as the excellent work by Claussen (1956), low density dependent natural enemies were segregated from high density dependent species. Where low density dependent natural enemies do not occur in an ecosystem, there may be a fruitful field for the introduction of such natural enemies from other areas or other countries. If the information on the economic importance of low density natural enemies is inadequate, applications with selective and unselective insecticides by a variable dosage sprayer on the lines of the previously discussed experiment, form a man-power saving method to ascertain whether important low density dependent parasites or predators are at work. If such experiments are combined with parasite rearing it can be quickly decided whether low density dependent natural enemies are absent or are merely restricted by hyperparasites. Observations on the plots treated by the variable dosage sprayer, are also made to ascertain at what minimum density of the host the natural enemy can still maintain itself, and finally, at what density of the host it can no longer effect control.

#### SELECTIVITY OF INSECTICIDES

##### PHYSIOLOGICAL SELECTIVITY

Sometimes the insecticide when brought in contact with the natural enemy does not harm it, although it kills the pest when taken up orally or in the gas phase. The phenomenon was first observed with nicotine vapour (Ripper, 1944) and is very noticeable with schradan (Ripper *et al.*, 1950).

Ahmed (1955) studied the effect of feeding predacious larvae with cadavers of the cotton aphids, killed with demeton and schradan. He confirmed previous finding that aphids killed by schradan are not themselves toxic; aphids killed with demeton by the dipping method were toxic to *Coccinella septempunctata* and to *Scymnus syriacus*, but both species survived when fed with aphids killed without external contact with demeton. Larvae of the Syrphid, *Sphaerophoria flavicanda*, and of the Ochthiphitid, *Leucopsis puncticornis*, were killed when fed on demeton- and schradan-killed aphids, but *Chrysopa vulgaris* could ingest demeton or schradan killed aphids with impunity.

Several hypotheses have been put forward to explain the physiological selectivity of schradan. Schradan is not directly toxic to insects; when ingested, the insect body converts it into a toxic substance. Based upon observations of the relative converting capacity of various tissues, O'Brien and Spencer (1953) suggested that susceptible insects convert schradan to the cholinergic oxidation product slowly within the nerve tissue, while in the non-susceptible species the conversion rate in the fat body of the insect is so great that little or no unconverted schradan reaches the nerve tissue and the short-lived oxidation product of the schradan is incapable of penetrating the insect lipoid nerve sheath. Casida (1955) and collaborators (Casida *et al.*, 1955) believe that the susceptibility of insects to schradan depends upon the sensitivity of the particular insect cholinesterase to the inhibition by the schradan metabolite, and demonstrated that the cholinesterase from the non-susceptible *Trialeurodes vaporarum* (Westwood) and *Blattella germanica* (L.) required 6.24 times as much oxide of schradan for inhibition as that of the house fly. Rhyania has been shown to be a good stomach poison for lepidopterous larvae (Clancy, 1955) without affecting the natural enemies of phytophagous species, and a similar selectivity has been produced by the above-mentioned coating of DDT and other insecticides with zein and other substances (Ripper *et al.*, 1948).

Unfortunately, no other compound with physiological selectivity has been reported in the literature; as schradan is slowly converted into a toxic substance in the insect body, one avenue to obtain new physiological selective insecticides lies in the further study of zoometatoxicity. In an analogous way new selective herbicidal compounds were synthesized by Wain (1955) through adding to herbicidal molecules side chains which only certain plants metabolize to the herbicidal rest of the molecule.

#### ECOLOGICAL SELECTIVITY

Several of the most useful systemics combine contact toxicity, vapour toxicity, and systemic effects (Corey *et al.*, 1953; David and Gardner, 1954; Davis and Sessions, 1953), and they are therefore not selective if applied as foliage sprays, for example, demeton or methyl-demeton are very toxic to Syrphid larvae, certain Coccinellids and to Hymenopterous parasites. Mathys (1954, 1955, 1956) found demeton as toxic to the fruit tree red spider, *Metatetranychus ulmi* and the predacious mite, *Typhlodromus sp.* as methyl-demeton. Some of the non-selective systemics can be used to achieve ecological selectivity in the form of seed treatment, soil application, bark treatment, or trunk implantation if the insecticide is unavailable to the natural enemies. This phenomenon was first demonstrated for dimefox and mipafox (Ripper *et al.*, 1951) and has since been confirmed in many cases (Hanna *et al.*, 1955). It is, of course, based on the translocation of the compound from the site of application to where the insecticide does not come in contact with the natural enemies.

The selective insecticides so far known are barely sufficient to allow examination of the practicability of integration in order to decide whether more intensive effort to discover or produce further selective insecticides is justified. Such research is important because most chemical manufacturers today hold that insecticides with a wide spectrum of insecticidal action are the desirable research objectives; a much larger volume of chemical production of an insecticide with a broad spectrum enables a better recoupment of the great development costs of a new insecticide and therefore allows the sale at a more reasonable price.

Selective insecticides must, for the same reason, be more expensive, but the success of schradan in England in comparison with the cheaper methyl-demeton shows that discerning growers are willing to pay more for a selective insecticide which enables an integration of biological and chemical control. Growers in England consider the biotic resistance of their crops to pests as a capital asset which they are trying to preserve and which they are not prepared to exchange for larger profits because of the spectre of resistance and a consequent recurrence of crop losses as experienced before the development of the newer synthetic insecticides.

If further experience should show that resistance develops with many phytophagous pests, and that resistance against one insecticide accelerates the development of resistance against other compounds, then it would indeed be urgent to find selective insecticides and to introduce them before resistance against other insecticides makes their use too difficult by stultifying their initial control effect.

# MAXIMUM NON-DAMAGING POPULATION DENSITIES OF THE PEST

As the integration of chemical and biological control aims at a re-establishment of a pseudo-equilibrium of the pest and its natural enemies at a low and not damaging population level of the pest, it becomes necessary to determine the correlation between the population density of the phytophagous species and the damage which it causes in order to arrive at the not damaging population density. There are several ways to obtain these data. Strickland has in a cooperative project with the National Agricultural Advisory Services in England correlated over a number of years the population densities of several crop pests with yields of marketable produce. By experiments at a great number of locations in different parts of the country, data of population density and crop yields were obtained and correlated (1954) from which the maximum not damaging pest population per unit area can be extrapolated. This will of course vary with the regenerative power of the plant, the climatic conditions of the season, economic and other factors. We are currently trying to obtain such correlation by further using the plots sprayed by the variable dosage sprayer, which have already been used to ascertain the mortalities of the pest and their natural enemies and pest population densities which are of interest for the maintenance of the natural enemies. At present such work is being carried out on cotton and on brassica crops, but it would seem to be applicable to many crops on which variable dosage sprayers can be used. The advantage of this method is that correlations of population density and yield are obtained in a systematic and labour-saving way.

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## DISCUSSION

A. P. RANDALL. Are there any systemic insecticides that are effective against tortricid larvae?

W. E. RIPPER. Dithio demeton and Thimet ® have been shown to kill some lepidopterous larvae.





Section  
on  
**MEDICAL AND VETERINARY ENTOMOLOGY**



*Section Editor*  
C. R. TWINN



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The Mating, Oviposition, and other Activities of Warble Fly Adults,  
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By J. WEINTRAUB

Science Service Laboratory

Lethbridge, Alta.

# The Development of *Loa loa* in *Chrysops silacea*, the Escape of the Infective Forms from the Head of the Fly, and the Effect of the Worm on its Insect Host

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## ABSTRACT<sup>1</sup>

Since Leiper's (1914) demonstration of the development of the filarial worm *Loa loa* in the tabanid flies *Chrysops silacea* and *C. dimidiata* several workers have recorded its development in *Chrysops* species, but only Kleine (1915) and Connal and Connal (1921, 1922, and 1923), whose work was exploratory in nature and incomplete, have studied the sites within the fly occupied by the developing worms. The studies of the development and migration of *L. loa* in *C. silacea* reported in this paper were undertaken with the object of extending the work of Kleine and the Connals.

The present studies were carried out in Liverpool with either wild or bred *C. silacea* (preserved in alcohol and glycerine), which had been fed in the Cameroons on human beings and monkeys whose peripheral blood contained the microfilariae of *L. loa*. Most of the flies studied were embedded in paraffin blocks from which thick and thin sections were cut, but a small number were dissected, without previous preparation, in alcohol and glycerine.

Developing worms were observed only in the cells of the fat body of the head, thorax, and abdomen and never in other tissues. Migrating forms were seen in the haemocoel of the abdomen, thorax, and head. The worms migrate from the abdomen into the thorax by way of the abdomino-thoracic junction and appear to travel through the thorax chiefly in the neighbourhood of the alimentary canal and the ventral nerve cord. The migration of the worms from the thorax to the head takes place through narrow haemocoelic channels in the neck and particularly via a relatively large ventral haemocoelic canal which terminates in the head in an extensive haemocoelic pool, the sub-cibarial haemocoelic space.

Although the emergence of worms from the mouthparts of the feeding fly has been observed on several occasions (Connal and Connal, 1922; Gordon and Crewe, 1953) the precise site of emergence has never been seen. A study of the mouthparts of *C. silacea* revealed several possible routes of escape of the worms from the head of the fly. One such route is via the labella, as occurs in *Wuchereria bancrofti* and its mosquito host. This appears unlikely to be an important route of escape, as only a few worms have been seen in the labium, and the kinking of this organ when it is retracted resulting in the partial occlusion of its lumen is likely to prevent the "streaming out" of the worms observed both by the Connals (1922) and Gordon and Crewe (1953). A second possible route is by way of the biting fascicle of the mouthparts and the membranes which bind them to the head capsule. The only element of the biting fascicle which appears to have a lumen sufficiently capacious to accommodate the infective stage of *L. loa* is the labrum, but the available space is very restricted and can only allow the passage of a few worms at a time. Thirdly, the worms may escape from the delicate labio-hypopharyngeal membrane (one of the boundaries of the sub-cibarial haemocoelic space) which connects the root of the labium to the underside of the hypopharynx. Worms have been observed in large numbers in the neighbourhood of this membrane and have been seen "stripping" it from the hypopharynx. It appears probable that most of the worms escape from the head by this route.

The mechanism of the emergence of the infective stages of the worms from the head of the fly seems to be as follows. When the fly inserts the biting fascicle into the skin the labium is retracted well into the sub-cibarial haemocoelic space and bent on itself, and the labio-hypopharyngeal membrane is put on the stretch. The fly then begins to feed and the rapid filling of the midgut with blood forces the fluid and gaseous contents of the abdomen forwards towards the thorax and head. It is conceivable that, with the steady increase in the intracranial pressure as this takes place, the active movements of worms lying in the sub-cibarial haemocoelic space may bring about rupture of the taut labio-hypopharyngeal membrane, resulting in a release of the worms from the head of the fly.

<sup>1</sup> For complete text see *Annals of Tropical Medicine and Parasitology* 52: 103-121. 1958.

In several flies infected experimentally by feeding on human volunteers and examined up to the 10th day following the infective meal, no damage to the vital organs was observed. However, in a fly killed 12 days after being experimentally infected there was considerable lysis of the dorsal flight muscles, with worms lying in the large cavity produced. In several wild caught flies with infections of undetermined duration worms were seen lying in the muscles of the thorax, in the retina and, in one case, in the brain. It seems possible (though there is as yet no direct evidence) that, if the infective forms of *L. loa* do not escape from the fly when they are "ripe", their subsequent peregrinations may cause them to penetrate and damage organs which they would not invade during the course of normal development.

Although the longevity of infected and non-infected flies which have not been offered a second blood-meal does not appear to differ significantly (Kershaw, Chalmers and Duke, 1954) a few experiments carried out by my colleague, Dr. W. Crewe, suggest that non-infected flies lived somewhat longer than infected flies when both groups were offered a second blood meal on the 11th day. It is possible therefore, that the rupture of the labio-hypopharyngeal membrane by worms emerging during the feeding of *Chrysops* may have an adverse effect on the fly. What effect damage to the muscles of the thorax, the ommatidia of the retina and the brain may have on the vital activities of the fly has not been determined. The answer to these and other problems will be elucidated only when more is known of the physiology of the fly and its reactions to environmental stimuli.

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### DISCUSSION

L. A. JACHOWSKI. 1. Do the infective larvae of *L. loa* actively migrate within *Chrysops*? 2. What food, other than blood, do *Chrysops* take in the laboratory? 3. Do infective larvae escape while feeding on solutions?

R. M. GORDON. (1) Not known, it is suggested that they do. (2) Under laboratory conditions the females take up juices and sugar water. In nature not known. (3) So far the results were negative, but "membrane feeding" has not been tried.

RUD. GEIGY. 1. How many stages does the worm undergo in the fly? 2. Where is the sausage-stage observed, in the muscles? 3. Are some muscles preferred to others for infection? 4. Is the flight of the infected fly inhibited because of the muscle infection, as observed in *Onchocerca*?

R. M. GORDON. (1) Still under investigation; at least three. (2) Not observed in muscle. (3) The worms appear to avoid penetrating the major flight muscles and are usually confined to the dorsal flight muscles. (4) Not known.

J. ALLEN SCOTT. Do you have any evidence as to how the infective forms enter the skin?

R. M. GORDON. All our observations suggest that the infective forms are unable to penetrate the intact skin of the host, and that they normally gain entrance to the deeper tissues through the "bore-hole" made by the vector.



# The Population Dynamics of Infection with *Onchocerca volvulus* in the Vector *Simulium damnosum*

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## ABSTRACT

In light or moderate infections of West African onchocerciasis the microfilariae are largely concentrated in the skin of ankles and calves and then of the buttocks, and few or absent in the trunk, head, neck, and arms. *S. damnosum* bites in greatest numbers on ankles and calves. In an advanced case of onchocerciasis, when the skin of ankles and calves becomes fibrosed, the microfilariae under the surface become fewer and transmission from host to vector is suppressed. Frequency distribution curves plotted from the numbers of flies taking up different numbers of microfilariae in cases of different intensities of infection are all log-normal, and by comparison of the geometric means it is evident that a case with moderate infection is a better reservoir than a lightly infected one, and that a case of advanced infection is the least effective reservoir. The longevity of the flies is not significantly influenced by the development of the microfilariae. The fly ingests the microfilariae with the blood-meal, and though many become included within the peritrophic membrane that is formed rapidly round the meal, some may escape. Those in the membrane presumably perish during digestion. The others develop in the thoracic muscles, and when fully developed are found in the head, antennae, and legs. The population of microfilariae ingested by the fly undergoes a progressive but irregular fall after the first two days of larval development. In the laboratory about one-tenth survive to the infective form, and, as about half the flies survive long enough to produce infective forms, about one-twentieth complete their development.

## INTRODUCTION

It is not at present possible by an analytical approach to build up a comprehensive mathematical model of infection with *Onchocerca volvulus*, for we are ignorant of many of the fundamental principles involved in the transfer of vector-borne helminth infections, and of this filarial infection in particular. Nevertheless, something is known of some of the factors involved in isolated steps in the transfer of infection, though at the moment such knowledge cannot be integrated.

### FACTORS AFFECTING THE INTAKE OF THE MICROFILARIAE OF *O. volvulus*

In infections of light or moderate intensity in the West African form of onchocerciasis the microfilariae are present in largest concentration in the skin of the ankles and calves and then of the buttocks, and are few or absent in the trunk, head, neck and arms. It is also known that *Simulium damnosum* bites in the greatest numbers on the ankles and calves. There is thus a correlation in space between the disposition of the microfilariae and the biting habits of the vector, which corresponds to the correlation in time between the diurnal appearance of the microfilariae of *Loa loa* in the peripheral blood of man and the daytime biting habits of its vector, *Chrysops silacea*. In an advanced 'burnt-out' case of onchocerciasis, however, when the skin of the ankles and calves becomes fibrosed, the numbers of microfilariae immediately under the surface of those areas decrease, and the mechanism of transmission from mammalian host to vector host changes from one of enhancement to one of suppression (Kershaw, Duke and Budden, 1954).

In addition to the pattern of the distribution of the microfilariae under the surface, there is also a pattern in the distribution in depth (Kershaw, Jamison, Nugent and Duke, 1956). In an infection of light intensity, the microfilariae are found in greatest numbers in the superficial parts of the subepidermal layer; in infections of moderate intensity they are still more numerous in the superficial parts, and very much more numerous in the deeper parts of the subepidermal layer; and in an advanced 'burnt-out' infection the microfilariae, though equally numerous, are concentrated deeply in the dermis. *S. damnosum*, in obtaining its blood-meal, erodes the skin down to the superficial layer of the subepidermal connective tissue. The intake of microfilariae is therefore dependent upon the concentration of microfilariae in this superficial layer.

### THE INTAKE OF THE MICROFILARIAE OF *O. volvulus*

The number of flies biting on the ankle and calf is roughly the same in infections of light and of moderate intensity, few obtaining their blood-meal from above the knee. There is no relation between the size of the blood-meal taken and the number of microfilariae ingested. In an infection of light intensity some flies fail to take up any microfilariae, but in cases of moderate intensity all flies take in some microfilariae. In an infection of advanced 'burnt-out' intensity few flies feed on the ankle, presumably because of the difficulty in obtaining a blood-meal from the fibrosed and thickened tissue; most of those that succeed in feeding take up no microfilariae, and those that do take them in take in but few. A larger proportion feeds on the calf, but again most flies take in no microfilariae, though a few take in moderate numbers. All those feeding on the thigh—and there are proportionately more in cases of 'burnt-out' infection—take in some microfilariae.

Frequency-distribution curves plotted from the numbers of flies taking up different numbers of microfilariae in cases of different intensities are all log-normal, and, by comparison of the geometric means, it is evident that the case with a moderate intensity of infection is a better reservoir of infection than a lightly infected case, and that a case of advanced 'burnt-out' infection is the least effective reservoir. (In one particular instance, an infection of moderate intensity was eight times more effective a reservoir than an infection of light intensity, and 16 times more effective than a 'burnt-out' case) (Kershaw, 1955, in press).

### FACTORS AFFECTING THE DEVELOPMENT OF THE PARASITE IN THE VECTOR

#### (1) THE SURVIVAL OF THE VECTOR *S. damnosum*

A graph of the survival of a wild-caught population of *S. damnosum* maintained in laboratory conditions follows a curve in which the rate of mortality increases with age in such a way that the logarithm of the rate is directly proportionate to age. The Gompertz function (the logarithm of the rate of mortality) is thus a sloping straight line given by the equation  $\log u = at + b$ , in which  $u$  is mortality,  $a$  and  $b$  are constants for the particular population, and  $t$  is time.

The longevity of the fly population is not significantly influenced by the development of the microfilariae of *O. volvulus* to the infective form. From experiments on longevity in laboratory conditions, however, we cannot infer that in nature the fly may not be adversely affected in other ways, such as in limitation of flight range or in the rate of reproduction.

#### (2) THE SURVIVAL OF *O. volvulus* IN *S. damnosum*

The microfilariae are ingested with the blood-meal, and, though many become included within the peritrophic membrane which is rapidly formed round the meal, others avoid inclusion and some may escape. Those retained in the membrane presumably perish during digestion of the meal; the others develop in the thoracic muscles, and when fully developed are found in the head, antennae and legs. We do not know of any factors which affect the survival of the parasite during and after its development in the thorax.

### THE DEVELOPMENT OF THE PARASITE AND ITS SURVIVAL IN *S. damnosum*

The population of microfilariae ingested by the fly undergoes a progressive but irregular fall after the first two days of larval development to the infective stage.

After ingestion by the fly, the numbers surviving in flies that die in the first two days remain of the same order as the numbers ingested, but in flies dying between the third and the sixth day there is a marked fall in the number of larvae which have survived to develop in the thorax; survival to the infective form on the seventh day or later is achieved with little further mortality. This progression is most readily seen in the flies that feed on a moderate infection. In flies feeding on light infections or on advanced 'burnt-out' infections though the numbers of microfilariae taken in are so much smaller that their fate can be followed with less certainty; the same trends are evident. In the laboratory, about one-tenth of the microfilariae ingested by the flies which survive long enough for infective forms to develop survive to the infective form, and, as about half the flies survive long enough to produce infective forms, about one-twentieth of the originally ingested microfilariae therefore complete their development to the infective form.

There is no evidence that the chances of survival of the microfilariae throughout development to the infective form are influenced by the numbers ingested. The relationship

between the effectiveness of light, moderate and 'burnt-out' infections as reservoirs of infection to the fly is reproduced and reflected in the population of infective forms which survive in the vector for transmission back to man.

Until we have greater knowledge of the fundamental principles involved in the transference of this infection in the field, particularly of the number of blood-meals taken by the fly during its life-time, of the time-intervals between the meals, of the survival of the fly in field conditions, and of the factors affecting it, the observations here recorded and the conclusions which may be drawn from them cannot be integrated as components of a comprehensive mathematical model.

That, however, is not a valid reason for deferment of quantitative study. The construction of mathematical models is of great value in experimentation and research, and a comparison of such models with nature (even though the models may be incomplete) reveals which assumptions should be rejected and which should finally be accepted in our concepts of epidemiology.

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### DISCUSSION

RUD. GEIGY. Are the worms able to pass through or behind the peritrophic membrane?

D. J. LEWIS. In most *S. damnosum*, as far as is known, microfilariae which are imprisoned by the peritrophic membrane never escape from it alive. Occasionally, in a heavily engorged fly, blood and microfilariae appear in the hind gut soon after a blood meal.



# Experimental Epidemiology with the Filarioid Infection of Cotton Rats

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## ABSTRACT

Several forms of filariasis affect man in the tropics, some severely. The vectors are mosquitoes or other blood-sucking diptera, depending on the species of worm. Filarioid infections are also known from a wide range of animals and birds, but little is known about their vectors. Important exceptions are mosquito-borne filariases of dogs and mite-borne filariasis of cotton rats. Cotton rat filariasis has made practicable attempts at quantitative investigations of the inter-relationships of host, parasite, and vector. Initial work dealt with cotton rats infected once only with different numbers of worms, the ensuing infections, their infectivity for the vector, and the efficiency of the mite as a vector. Investigations were then developed on the more complex results of re-infection, artificial epizootics being arranged under laboratory conditions. Prolonged exposure to re-infection resulted in cotton rats of subnormal weight, associated with considerable infections of adult worms. But few or no microfilariae occurred in the blood-stream, thus disrupting, or jeopardising, the continuity of transmission. With intermittent transmission, the cotton rats were of normal weights, considerable adult worm infections were again evident, and the microfilarial infection of the blood was extremely high and persisted so for months. Transmission is assured, although less intensively than might be supposed. These two patterns are contrasting cases of an infinitely variable quantitative relationship between the host, parasite, and vector. They suggest that marked seasonal transmission, as opposed to all-year-round transmission, could be important to the apparent incidence and the epidemiology of, at least, cotton rat filariasis if not other infections in other hosts.

## INTRODUCTION

Several forms of filariasis, due to infection with several different species of helminth, affect man in the tropics and the vectors are mosquitoes, ceratopogonids, simuliids, or tabanids according to the species of worm concerned. There is an extensive literature dating back to the turn of the century, most of it on the mosquito-borne filariases. In the past decade notable advances have been made in our knowledge of these infections and, particularly, of the other human filariases transmitted by the other biting flies. Research on filariasis was, however, long handicapped by the lack of an infection in a suitable laboratory animal. This statement is not denying valuable researches in the laboratory with human infections and their vectors, with mosquito-borne filariases of dogs and, more recently, with monkey filariasis. When, however, ten years ago it was proved (Williams and Brown, 1946) that cotton rat filariasis was transmitted by a blood-sucking mite (for long known as *Liponyssus bacoti*, then changed to *Bdellonyssus bacoti* and currently named by some as *Ornithonyssus bacoti*) fresh opportunities were available for experimenting with filariasis. Both the mammalian host and the arthropod vector are readily bred and kept as clean strains in the laboratory and the host infected as required with its filarioid parasite, *Litomosoides carinii*, by passage through the mite.

## SIMPLE INFECTIONS

The writer, first concerned with the material as an entomologist, wished to break away from the uncertainties of empirical methods for obtaining transmission by interchanging infected and uninfected cotton rats in artificial mite-infested nests, successful enough as these could be (Bertram *et al.*, 1946; Hawking and Sewell, 1948; Scott, 1946; Scott, *et al.*, 1947; Williams, 1948). First, the vector's biology was worked out and means of handling it. The procedure for achieving transmission to cotton rats was briefly as follows: About, say, 300 female mites were released on an infected cotton rat in a suitable cage for 24 hours. The rat would destroy some of them and some would not feed fully enough. The well-fed mites were stored for a fortnight at 25°C., with two blood meals on an uninfected rat to improve their survival. At the end of that time infective worm larvae had developed in the mites which could then transmit the infection by feeding on a fresh mammalian host. Generally, 40 or 50 of the mites were dissected to determine the



infection rate and the number of infective worms in each infected mite. The latter facts were reduced to two additional measures of infection, viz. the mean number of worms per mite and the maximum number of worms in any one mite. This provided basic quantitative information enabling estimates to be attempted of how many worms were likely to be transmitted to each of several cotton rats on which, say, 20 mites of this same series were released to feed and effect transmission. Provided the infection rate in the mites was less than about 50 per cent quite good agreement was found between these estimates and the numbers of worms subsequently found in cotton rats at autopsy or post mortem. Above 50 per cent, some of the mites had large or very large infections—up to about 70 infective larvae in a mite. This was statistically unsuitable for acceptably precise transmission.

However, using suitably lightly infected mites, useful quantitative information was obtained. The sexes of the worms were transmitted in nearly equal numbers. They grew to maturity in the pleural cavity whence microfilariae from the mature females escaped to circulate in the blood of the cotton rat and await the chance of ingestion by the vector-mite. A worm of each sex was sufficient to give a microfilarial infection of the rat's blood, though there would be very few present. With an increase in the numbers of the adult worms the microfilarial count also increased but not proportionately. At quite a low adult worm count of, say, 12 to 20 worms of each sex the microfilarial count is already being suppressed. It is difficult to get in these simple infections microfilarial densities much beyond about 700 to 1000 mf. per cu. mm. which is, as we shall see, quite a low level for this infection. Such counts are obtained at the peak period of a rat's microfilaraemia which occurs about the fifth month after microfilariae first appear in its blood. From the peak the count gradually drops to nearly zero or zero. A year or eighteen months after the day of transmission a cotton rat may well have outlived its infection, the microfilariae having disappeared and adult worms being represented only by dead, encapsulated fragments. This limit to the microfilarial count at the peak of infection was puzzling as it was known from the early years of these studies that some cotton rats infected in the field and then kept to establish the strain in the laboratory had microfilarial counts of up to 3000 mf. per cu. mm.

## SUPERINFECTIONS

### (a) CONTINUOUS EXPOSURE TO INFECTION

It was clear that these so-called simple infections induced on a single day in the laboratory bore little resemblance to the infections which could occur in nature where a cotton rat might be bitten repeatedly by infective mites over a year or two at least. Moreover the numbers of worms it would be receiving at any one moment were virtually limitless. The possible variations in the amount and frequency of transmission were tremendous. The immediate answer seemed to be to set up some kind of artificial mite-infested nest shared by infected and uninfected cotton rats and to keep them together for a long period of time. The ideal of keeping strictly to quantitative methods of transmission at each repeated exposure of a cotton rat to infection was impracticable for several reasons. It was tried. A special nesting method was devised (Bertram, 1954) in which cotton rats were exposed to the continuous risk of repeated re-infection for up to six consecutive months. Periodic films were taken of the blood of the cotton rats during their exposure to infection and for up to a further six months after leaving the nest. The results were at first surprising (Bertram, 1953) though retrospectively they seem logical. Despite the long time in the mite-infested nests the microfilarial counts were commonly very low or the blood was quite negative. Yet mites had been numerous in the nests, in some nests extremely so. It could have been that they were dying off before becoming infective but this seemed unlikely. In due course, post mortems and autopsies of the cotton rats were completed and it was then clear that repeated re-infection had, in fact, occurred to the extent that the adult worm infection had been, so far as one could count the tangled masses and encapsulations, quite heavy. Counts in a single rat like 79 ♀♀ and 109 ♂♂ plus encapsulated, uncountable, worms were obtained and it could be shown that the worms were undersized and producing microfilariae hardly at all or not at all. The low and negative blood infections were explicable. This was interesting but still failed to account for microfilarial infections of 2–3000 mf. per cu. mm. known to be possible. One reason for wanting to create such heavy infections in the cotton rat was to test their significance as regards infecting the mite. This aspect of the work still could not progress.

### (b) INTERMITTENT TRANSMISSION

There were, however, indications from one rat out of these nests as to how one might obtain very high microfilarial counts. This animal had, besides over 225 adult worms in the pleural cavity, 4 female worms and one male in the peritoneal cavity and its microfilarial count was about 1000 mf. per cu. mm. on two occasions separated by five months. The pleural worms were undersized and poorly productive but those in the peritoneal cavity were long, robust and highly productive. A second point was that this rat was originally a simple infection and after four months had been put into one of the mite-infested nests to infect the mites. This was an intermittent transmission, which seemed worth further investigation as a means of inducing high microfilarial counts.

Using the same nesting technique, cotton rats were now exposed to transmission on several occasions for 10 to 21 days at intervals of a few months. This time expectations were fulfilled. Animals exposed to three and four exposures in this intermittent way developed microfilarial infections of 2500 to 3000 mf. per cu. mm. which still continued about this level in one instance for over a year after the blood was first positive for microfilariae. In these infections successive populations of adult worms had been able to reproduce successfully and maintain a high microfilaraemia in the rat's blood. In the pleural infections such as, for example, 93 ♀♀ and 123 ♂♂, the females were of good average size and produced microfilariae in large numbers. Some animals harboured a few adult worms in the peritoneal cavity but others did not. There was no doubt that intermittent transmission at considerable intervals resulted in very high microfilarial counts, although it is not easy to be certain how important peritoneal invasion by adult worms is in giving this result.

### MITE INFECTIONS

Some tests were now possible on how much mites would be infected if they fed on cotton rats with such high microfilarial counts. So far, there is no confirmation of infection rates as low as about 10 per cent, such as was obtained in some early work (Bertram, 1950). It is, however, difficult to compare early findings with more recent results as the level of infections in the mites obtained later in this work has tended to be higher than in the earlier studies. This may be due to changes in technique but this is not certain. However, confining ourselves to more recent results it can be shown that very heavy microfilarial infections in the cotton rat are by no means proportionately infective to the mites. For example, cotton rats with blood infections of 375 mf. per cu. mm. and 6989 mf. per cu. mm. gave infections in mites of, respectively, 95.5 per cent and 91.3 per cent and mean numbers of worms per mite of 21.3 and 19.9 worms. The maximum infections were similar, viz., 55 and 59 worms. This result, in fact, shows the more heavily infected cotton rat to be barely as efficient as the lightly infected animal as an infector of the mites. Most results, in fact, show a heavily infected host as certainly rather more successful in infecting mites but, the point is, never to a degree proportionate to its high microfilarial count. The principle has already been observed in some of the earlier work with cotton rats with microfilarial densities of less than a 1000 mf. per cu. mm. (Bertram, 1950). Cotton rats with very high microfilarial counts are not, then, remarkably better sources of mite infection.

It remains to note that cotton rats exposed to long continuous risk of repeated re-infection resulting in nearly negative or negative blood, despite considerable adult worm infections, were about half normal weight, lethargic, and their fur stood erect. Cotton rats with very heavy adult worm and high microfilarial counts following intermittent transmission at widely spaced intervals were of normal weight and appeared fit.

### CONCLUSION

In conclusion, it may be suggested from these results in experimental epidemiology (or epizootology) with this infection that the natural counterparts of continuous exposure to transmission and to intermittent transmission in the laboratory may be something approximating to all-year-round transmission on the one hand, and to seasonal transmission on the other. It seems an interesting principle to keep in mind in connexion with the epidemiology of this and other filariases, including those of man. Not only may the density of the microfilarial infection of the mammal's blood be so markedly different, depending on the pattern of transmission, but the percentage incidence of positive bloods in a population could be misleadingly low under conditions of intense continuous transmission. Coupled with this,

effects on the health of the vertebrate host may only be appreciable if transmission is continuous rather than intermittent. There is some evidence of such relationships in the human filariases.

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# Maintenance of Tropical Rat Mites and the Quantitative Transmission of Cotton Rat Filariae

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## ABSTRACT

Colonies of tropical rat mites are maintained on a continuous basis on white rats housed in containers which prevent the egress of mites. Provision is made for inspection of the rat and for supplying food without opening the container. At the end of three weeks the rat is transferred with the attached mites to a new unit. An apparatus involving a heating lamp removes the mites from the material in the old unit. The nymphs so obtained can be returned to the rat to hasten the development of the colony. The adults are held at constant temperatures for one week, fed on an infected cotton rat, again isolated and held at constant temperature and humidity for 2 or 3 weeks. They are dissected to remove the infective larvae which are counted and introduced into a subcutaneous pocket or to the abdominal cavity of experimental rats. Satisfactory criteria of immunity due to existing infections are the mean length of the worms 24 days after infection as compared to the length of worms from previously uninfected control rats, and the percentage of worms in the two groups which have started the fourth molt by this time.

Methods for maintaining colonies of tropical rat mites (*Bdellonyssus bacoti*) and for infecting them with *Litomosoides carinii*, the filarial worms of the cotton rat, have been described by several authors including Williams (1946), Bertram *et al.* (1946), Hawking and Sewell (1948), and Philip and Hughes (1948). The methods described are the result of several modifications since the original methods were introduced in 1945. For our purposes they have proved more suitable than other methods especially since large numbers of mites can be produced with the least labor.

We raise the mites on white rats since they are cheap and large enough to support large colonies. It may be interesting to note in passing that the mites multiply most rapidly on rats which have been used for some time and are anemic and debilitated. I do not know why this is true. The rat is placed in a metal tank described by Scott *et al.* (1947). The top of the tank is surrounded by a moat containing oil to prevent escape of the mites. An inch of sawdust is placed in the bottom of the tank to absorb moisture and is covered with wire screen to prevent the rat from burrowing. On top of the wire there is a layer of hay to allow the mites to crawl out of the damp sawdust. The sawdust is previously heated in an oven to 50°C. and the hay autoclaved for 5 minutes to kill predaceous mites. A wire cover hangs inside the top of the tank and supports a conical wire feeding basket. A pan beneath the basket catches crumbs and spilled food. At first we had trouble with cheese mites. By starting with clean materials and changing the crumb pan daily we apparently eliminated them from the building and now do not empty the pan until a satisfactory colony has developed. When a dozen or so mites are seen on the rat, the colony is usually large enough to remove. In any case the units are not allowed to run more than 3 weeks since the colonies will diminish after this time. The rat is then removed to a clean unit with whatever mites are on it. If it is desired to build up the colony faster, the nymphs recovered from the unit are put into the new unit.

The mites are removed from the bedding material by the use of a modification of an apparatus described by Scott (1948). The hay from the colony unit is placed in the bottom of a metal trough. This trough is 31 in. long, 7 in. wide and 4 in. deep and surrounded by a shelf 1½ in. wide and one-half inch below the top of the trough. An infrared reflector heat lamp (General Electric) is suspended from a trolley on a track over the center of the trough with the bottom of the bulb 6 in. above the top of the trough. It is pulled along the track at the rate of one-half inch per minute by an electric clock motor. The mites move ahead of the heat or crawl over the edge of the trough onto a shelf around the top. The shelf and the bottom of the trough are covered with asbestos board since the heat is transmitted by the metal more rapidly than the lamp moves. Around the outside edge of the shelf there is a brass tube in which there is a heating wire insulated by glass tubing or Teflon high temperature spaghetti tubing. Number 28 nichrome wire in series with a 75 Ohm resistor on 110 volt current develops approximately 0.4 watts per inch. This



amount of heat is sufficient to repel the mites at  $\frac{1}{4}$  inch, but will not cause a burn if touched. The apparatus does not need constant attention, but every 15 or 20 minutes the mites which have crawled out are picked up in a bottle attached to a suction pump.

The mites are then dumped from the bottle onto a sheet of white paper inside a ring of brass tubing. To keep the tubing hot it contains "Mallory Yard-Ohm resister" inside of insulating loom. This resister can be purchased in various resistances to provide approximately 0.5 watts per inch on 110 volt current. The adults and nymphs are picked up in separate suction bottles. The adult mites are held at a constant temperature of 22°C. for about a week when they will again be hungry.

Several hundred hungry mites are dumped onto the back of a cotton rat infected with filarial worms. This rat is confined in a small cage which rests on a layer of hay and sawdust in a small tank provided with an oil moat. After a day or two the cage is suspended from the top of the tank to allow the mites to drop off the rat into the hay. Nearly all the mites will drop off within 24 hours, at which time the cage is removed to a clean tank and the hay and sawdust processed as before.

The mites recovered from the cotton rat are placed in a heating ring and those that are well engorged are drawn by suction into a pyrex glass tube 22 mm. in diameter and 10 cm. long. This tube is then covered at both ends with 12 XX bolting silk and inserted into a wide-mouth bottle containing a little water to keep the humidity high. The unit must be kept at a constant temperature or dew will form, trapping the mites and killing them. They can be kept successfully for 3 or 4 weeks without additional feeding.

Nearly all of the larval filariae develop to the infective stage in about 18 days. They are then teased apart under a dissecting microscope in half strength Tyrodes solution. The larvae are separated from the debris and transferred with a fine pipette to a cover glass which is supported on small blocks. When conditions are favorable, 200 larvae can be accumulated by two people in a half hour. The larvae are examined carefully and any which have been damaged, or which have not reached the infective stage, are removed. The others are then counted.

To infect a rat an incision is made under ether in the skin of the flank and a subcutaneous pocket formed by loosening the skin around the incision with a blunt instrument. Sutures are then inserted and the pocket held open while another person pours the larvae from the corner of the cover glass and rinses it with a few additional drops of solution. While the sutures are being tied, the cover glass is again examined to see if any larvae remain. In this way precise quantitative infections are possible.

Our studies on these filarial worms have been concerned primarily with various aspects of immunity. Among the criteria of immunity are a reduced percentage of worms developing, retardation of growth of the worms and encapsulation or early death. For these studies it has been necessary to develop these methods of providing a constant supply of infected mites. Thus we can schedule successive infections, each of which is produced by the simultaneous introduction of known numbers of infective larvae.

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## DISCUSSION

P. T. JOHNSON (Miss). Were the mite colonies obtained from *Rattus* or *Sigmodon*? *Rattus* is an introduced rodent (to America) and it seems odd that a mite normal to *Rattus* would be the vector of a filarial infection of a native American rodent *Sigmodon*.

J. ALLEN SCOTT. Originally we used cultures of mites from both *Sigmodon* and *Rattus* (white laboratory rats). We found no differences in infectivity and have used only the strain from *Sigmodon* for several years. We find these mites on *Sigmodon* in Galveston County with great regularity.



# *Culex bitaeniorhynchus* as Vector of *Wuchereria bancrofti* in New Guinea

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## ABSTRACT<sup>1</sup>

During a recent survey in a New Guinea Papua village, three culicine mosquitoes, caught in houses were found naturally infected with mature forms of *microfilaria bancrofti*. The species most frequently encountered was first a *Culex bitaeniorhynchus*-like mosquito, second *C. annulirostris*, and third *Taeniorhynchus uniformis*. Anopheline mosquitoes were not caught in the village. The surprising circumstance is that this *C. bitaeniorhynchus* which I know only as a species that never attacks man was found in great numbers indoors and bit man indiscriminately. Morphologically the specimens agree rather well with the characters as given by Edwards in different publications, though they are distinctly darker in color.

It has been known that *C. bitaeniorhynchus* is a variable species. Edwards, however, recognized only two varieties: *ambiguus* and *tenax*. Does *C. bitaeniorhynchus* represent a complex or subgroup as we have seen in New Guinea for *Anopheles punctulatus* and elsewhere for *A. leucosphyrus*, *A. umbrosus*, *A. hyrcanus*, and probably other anophelines? Taking the variability for granted there still is the totally different attitude towards man, in so far as the New Guinea species not only enters houses, but feeds on man regularly.

<sup>1</sup>The paper was published in *Documenta de Medicina Geographica et Tropica* 8(4): 375-379. 1956.



# Mite Transmission of Haemogregarines and Filariasis in Captive Snakes

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## ABSTRACT

Blood-smear surveys of the snakes housed at Lincoln Park Zoo, Chicago, revealed a consistently high incidence of blood protozoans, genus *Haemogregarina*, and some cases of microfilarial infection. In cages containing several snakes, the haemogregarines were rarely restricted to a single host and snakes raised from birth in the zoo were also infected, indicating the presence of the vector on the premises. In every case in the zoo collection, the snake mite, *Ophionyssus natricis* (Gervais), was found associated with the haemogregarine infections. Mites that fed on the blood of haemogregarine-infected snakes consistently had concentrations of sporozoite-like organisms in the wall of the midgut, whereas those that fed on uninfected snakes lacked them. The villi of the small intestine and the stomach of snakes with haemogregarine gametocytes in their blood contained numerous spindle-shaped parasites, which appear to be developing trophozoites. Transmission experiments with the snake mite are in progress. In the first survey in May, 1955, the reptile collection showed an infection rate of 42.4%; one year later the rate was 23.1%. In the intervening year, efforts by zoo personnel significantly reduced the mite populations. *Microfilariae*, ingested with a blood meal, underwent developmental changes in the mites. Evidence suggests that *Ophionyssus natricis* may also serve as a vector.





# Mortalité chez les Culicidés infestés par *Dirofilaria immitis* et *Wuchereria bancrofti*

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## RÉSUMÉ<sup>1</sup>

Ces recherches ont été faites à Hanoï (Tonkin) en vue de comparer les effets chez le vecteur et hôte intermédiaire du développement de *D. immitis* et *W. bancrofti*. On a utilisé les espèces suivantes: *Aedes aegypti*, *A. albopictus*, *Culex fatigans*, *Armigeres obturbans* et *Anopheles hyrcanus var. sinensis*.

A 28–32° C., la mortalité est considérable pendant les cinq premiers jours, c'est-à-dire pendant la période d'invasion des tubes de Malpighi par les microfilaires de *D. immitis*. Les spécimen restant peuvent survivre jusqu'au 40ème jour. Le degré de mortalité est aussi fonction de l'importance de la microfilarémie du chien. A 18–22°C., la mortalité est réduite les 5 premiers jours, même si la microfilarémie est élevée et la survie est plus longue (50 jours en moyenne).

Il y a évidemment, à 28–30°C., une différence considérable avec l'effet des microfilaires de *W. bancrofti* qui effectuent tout leur développement dans les muscles du thorax. La mortalité est faible pendant les 5 premiers jours, et la courbe reste basse après cette période. La survie peut atteindre 70 jours.

La migration des larves mûres du 3<sup>e</sup> stade infestant dans la tête et dans la trompe n'ont d'effet sur l'accroissement de la mortalité à cette période ni dans le cas de *W. bancrofti*, ni même dans celui de *D. immitis* où la formation de ces larves volumineuses a une action destructrice sur l'organe excréteur.

Dans le cas de *D. immitis*, il n'y a aucune relation entre l'aptitude d'un culicidé à permettre le développement larvaire de *D. immitis* jusqu'au stade infestant, et le degré de mortalité. Ainsi chez *Armigeres obturbans* qui est réfractaire, et chez qui le développement des microfilaires est rapidement inhibé, la mortalité est plus élevée en raison de l'invasion précoce et massive des tubes de Malpighi.

Le degré de mortalité pendant les cinq premiers jours de l'infestation par *D. immitis* est aussi en rapport avec l'intensité du métabolisme sexuel chez la femelle. Ainsi chez les femelles non fécondées, la mortalité est beaucoup moins élevée pendant cette période, et la survie générale est plus longue que chez les femelles fécondées.

## DISCUSSION

C. B. PHILIP. Using clearing technique, did mortality due to microfilariae appear to coincide with migration from Malpighian tubules? Curves on charts suggested period of about 5–10 days after feeding.

H. GALLIARD. Mortality is not related to migration out of Malpighian tubules. Period of migration is after 9 days and the heavy mortality takes place during the first five days.

J. FRAGA DE AZEVEDO. We know that there exists a close relation between the number of microfilaria and the mortality of the mosquitoes. Can you tell me if this mortality is the result of a toxic or a resistance action or if it is the result of other factors?

H. GALLIARD. In the case of *D. immitis* it is obvious that it is the penetration in the Malpighian tubules by microfilariae that causes death by inhibition of the excretory function.

L. A. JACHOWSKI. I am very interested in your technique of fixing and clearing infected mosquitoes. What is the appearance of the worms after clearing with lactophenol? What is the reference to which you referred?

H. GALLIARD. Larvae are seen by refringence. The inner organs are only visible if alcohol is substituted for chloral lactophenol. The reference is Procédé de Recherche des M. F. de *W. bancrofti* chez les moustiques desséchés. Ann. de Parasitol. XIV, No. 5, 1936

<sup>1</sup> A été publié dans Zeitschrift für Tropenmedizin und Parasitologie (Stuttgart) 8(4), 1957.



# Biology and Control of Simuliid (Diptera) Vectors of Onchocerciasis in Central America<sup>1</sup>

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## ABSTRACT

The study of black flies in relation to onchocerciasis was carried out at San Pedro Yepocapa, Guatemala. Of 6 species that readily attack man, *Simulium* (S.) *ochraceum* and S. (S.) *metallicum* are the most important vectors, and S. (Lanea) *veracruzianum*, S. *haematopotum*, and S. (Notolepria) *exiguum* are probably important in the upper and lower limits of the disease zones. The maximum density of *ochraceum* coincides with the greatest endemicity of the disease but the most numerous man-biting species is *metallicum*. S. *ochraceum* breeds principally in infant streams concealed by dense vegetation, and *metallicum* in a variety of habitats. S. *ochraceum* prefers the upper part of the body whereas *metallicum* attacks principally the lower limbs. Normal females travel at least 10 miles and can live 85 days in nature; infection seems to reduce flight and longevity. Because of the flight range and longevity and the dispersion of the breeding areas of *ochraceum* and *metallicum*, and the impracticality of using aircraft to treat these areas, control was limited to tributaries and main streams (about 1,500) in an experimental area of about 75 sq. mi. For most *ochraceum* breeding areas, rivulets usually not exceeding 200 ft. long and volumes less than 100 gal./min., a single application of DDT emulsion at 0.1 p.p.m. for 3 min. eliminated larvae. This dosage was also suitable for streams with volumes up to 4,500 gal./min., in which most *metallicum* were found. In larger streams, 0.1 p.p.m. for 60 min., or 2 p.p.m. for 3 min. were used. In general, the greater the stream-volume at the point of treatment, the farther the insecticide was effective.

## INTRODUCTION

In the Americas, many arthropod-borne diseases are of far greater importance to human welfare than onchocerciasis because of their widespread distribution and acute clinical picture, but few of these diseases are so apparent by their outward manifestation, or play such a marked role in the economy of the people afflicted, as does onchocerciasis in its endemic foci.

Onchocerciasis, as a disease caused by a specific worm parasite, has been known since 1893 when it was described from the Gold Coast in Africa (Leuckart, 1893). It was first reported from Central America in 1915, when Dr. Rodolfo Robles (Calderón, 1917; Robles, 1919) isolated the adult worms from nodules taken from a child living on a coffee plantation in Guatemala. It has recently been estimated that 35,000 persons in Mexico and 25,000 persons in Guatemala are infected. The total population of Guatemala is only approximately 2,500,000. As recently as 1948, the disease was reported for the first time from an area in the northwest of Venezuela (Potenza *et al.*, 1948), where almost 50% of the population was infected. The extent of the zone is not known. Reports of the disease from Columbia and Ecuador have not yet been authenticated. In Central America, as in West and Central Africa, infection rates in some areas reach 80 to 100% of the population (WHO, 1954). The disease is of great social importance in view of the high rate of ocular complications which may result in total blindness, affect the visual acuity to such a degree as to incapacitate the individual from any gainful work, or greatly reduce his working capacity. In Guatemala, 67% (Riveroll Noble, 1949) of those infected have ocular manifestations and about 5% of these are totally blind. Since coffee production, which requires rapid picking of the coffee berries by field labor, is the backbone of the economy throughout most of the onchocerciasis zone, it is impossible for afflicted individuals to engage in normal work and they soon become economic charges on their already poverty-stricken families.

Since Robles' hypothesis (1919) concerning the transmission of human onchocerciasis in Guatemala by *Simulium* flies, and Blacklock's experimental proof that *Simulium damnosum* transmits the disease in Africa (1926a and b), it has been generally accepted that species of Simuliidae are the vectors. The investigations of Strong (1931a and b), Hoffmann

<sup>1</sup>Figs. 1 to 6 and 9 either taken directly, or adapted, from Dalmat, 1955; Fig. 8 taken from Dalmat, 1950, and Fig. 10 from Lea and Dalmat, 1955.

<sup>2</sup>Laboratory of Tropical Diseases.

(1930a and b), De León (1940a and b), Vargas (1948), Gibson (1951), and Dalmat (1955), all working in Guatemala or Mexico, have corroborated the evidence of Robles and Blacklock. Upon epidemiological grounds, *Simulium ochraceum*, *S. metallicum*, and *S. callidum* have been considered the probable vectors. All three have been found naturally infected with *Onchocerca* larvae. Based principally on the apparent coincidence of the geographic distribution of *ochraceum* with the endemic regions of onchocerciasis, together with its anthropophilic nature, this species has been adjudged the principal vector.

It is true that *Simulium* species do abound in all regions with endemic onchocerciasis and that transmitting species would necessarily have to be ones that attack humans. However, during the past 10 years, it has been shown that there are several species of black flies in Guatemala and Mexico that bite man and can become infected in nature or experimentally (Gibson and Dalmat, 1952; Dalmat, 1955; and Vargas, 1948). It is therefore necessary to examine the available data on these species so as to be better able to judge which are the vectors.

### ONCHOCERCIASIS ZONES

In Guatemala, the principal onchocerciasis zone is situated from about 1,500 feet to 5,000 feet along the Pacific versant of the foothills of the Sierra Madre, which traverses the entire country in a general southeasterly direction. The zone extends as a 75-mile-long band (500 sq. miles) from the volcano San Pedro in the west to Tecuamburro in the east (Fig. 1). Along the Pacific slope of the Sierra Madre, which rises out of the coastal plane like a solid wall, there is a pronounced dry season extending from November through April and a wet season from May through October, the latter being caused primarily by the more local south-southwest winds from the Pacific which blow somewhat irregularly from May through October. This zone receives large amounts of rainfall, primarily during the 6-month rainy season. Within any one region and at great proximity, may be found startling variations in climatic conditions due to the particular location of the area, the direction of the slope, or the degree of exposure to prevailing winds.

A second zone of onchocerciasis, about 85 square miles in size, exists in the northwestern part of the country in the Department of Huehuetenango, contiguous to the state of Chiapas in Mexico. This endemic region, like the Chiapas zone in Mexico, is situated in the eastern part of the Valley of Chiapas, between two branches of the Andean chain, rather than on the Pacific slope of the more southern branch. To date, the actual extent of the onchocerciasis zones has not been clearly defined. Since all knowledge of them on the part of the health authorities of Guatemala has been secured from visiting plantation areas where onchocerciasis has been known for years and where nodules have been extirpated surgically, it is impossible to state whether or not there are many peripheral areas that should be included and whether or not entirely distinct zones may exist. No systematic survey has ever been carried on.

The majority of my remarks will be confined to the larger endemic zone of Guatemala, hereafter referred to as the Yepocapa Zone, since it was in the town of San Pedro Yepocapa, in the Department of Chimaltenango, that our central field laboratory was established. The town is situated at the highest elevation at which the disease is found and approximately midway between the western and eastern limits of the zone. Of 8,200 inhabitants in the town and outlying areas, about 75% live on, and are employed by, the large coffee plantations. Ninety-five percent of this rural group are Mayan Indians. The town is situated on the western slopes of the volcanoes Acatenango (12,992 feet) and Fuego (12,730 feet) at an elevation of 4,850 feet. Looking southward, can be seen gradually descending chains of foothills, and the Pacific ocean can barely be distinguished at the horizon some 40 miles away. The location of Yepocapa is rather unique in that it is fully exposed to the moisture-laden winds of the Pacific, to the more local conditions effectuated by the overlooking eastern volcanoes which serve as a barrier, and to the downwinds that cross over these volcanoes from the northeast. The interaction of these factors result in isolated conditions of precipitation, temperature, and winds.

The weather of the Yepocapa region can most easily be appreciated from an examination of the meteorological data, collected over a 5-year period from August 1, 1947 through July 31, 1952, which have been summarized in Table I. It will be noted that the mean monthly maximum temperature varies slightly throughout the year and the mean monthly minimum also varies little, although there is a slight reduction in temperature during Decem-



TABLE I—Meteorological Data—San Pedro Yepocapa, Guatemala. (All Monthly Figures are Expressed as Means for 5-year Period, 1947–1952).

Meteorological factor	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Average 5 years
Mean monthly maximum temperature (°C)	26.1	25.2	25.1	25.8	25.6	25.8	26.0	25.7	26.7	26.7	25.7	26.4	26.0
Mean monthly minimum temperature (°C)	13.3	12.3	12.6	12.2	11.8	11.6	10.3	12.0	12.6	12.9	12.9	13.1	12.3
Mean monthly temperature (°C)	19.7	18.7	18.8	19.0	18.7	18.7	18.2	18.8	19.7	19.8	19.4	19.8	19.1
Mean relative humidity	88	90	88	89	84	82	80	79	80	86	89	83	85
Precipitation (mm.)	395	730	473	90	15	13	15	65	115	378	494	393	3168
days	17	24	16	5	1	1	2	3	8	20	22	19	139

ber, January, and February. The reduction in minimum temperature is reflected in a slight seasonal drop in the mean monthly temperature during the same months. This is the middle of the "cool" or "dry" season. Also, as would be expected, there is a corresponding drop in precipitation and relative humidity which is more noticeable, and extends over a longer period, than does the reduction in temperature. Beginning in October or November, a period of stronger winds is also recorded, this being caused by the winter trade winds blowing from the northeast. It can be said that the general climate of the Yepocapa region is mild and rather constant, the only radical changes being in rainfall and relative humidity.

### ANTHROPOPHILIC SPECIES

In Guatemala, the species of black flies found to attack man readily, and therefore worthy of consideration in the transmission of onchocerciasis, are *Simulium* (*Simulium*) *ochraceum* Walker, *S. (S.) metallicum* Bellardi, and *S. (Lanea) callidum* Dyar and Shannon, *S. (L.) haematopotum* Malloch, *S. (Notolepria) exiguum* Roubaud, and *S. (L.) veracruzianum* Vargas, Martínez and Díaz. As stated previously, *ochraceum* has consistently been considered the principal vector of onchocerciasis in Guatemala and Mexico based on its decidedly anthropophilic habits and because of its distribution in relation to that of the disease. Most epidemiological reports claim that the distribution of *ochraceum* coincides with that of onchocerciasis. It should follow that the species does not exist, or is only infrequently found, outside of the onchocerciasis zones, and that the distribution of other anthropophilic species is not of this pattern. Our findings showed that *ochraceum* is widely distributed in Guatemala, both inside and outside of the endemic areas, but outside of the zones it is found in greatly reduced numbers and does not seem to be such an avid human biter. *S. metallicum* and *S. callidum* are also widespread both within and outside of the disease zones, but their optimum range is much more extensive than that of *ochraceum*. Since *callidum* is found only sporadically throughout its entire range, and never in great numbers, it can probably be disregarded as an important transmitting agent. *S. exiguum* and *haematopotum* are primarily found at the lower regions of the zone and below it, while *veracruzianum* is most abundant in the upper limits and above the zone. Since the maximum *ochraceum* density does coincide with the areas of greatest endemicity of onchocerciasis, it would appear that this species might well be the most important vector.

In collections of about 70,000 adult flies from human bait in the endemic zones, 65% were *metallicum*, 30% *ochraceum*, 4% *callidum*, and the remaining one percent distributed amongst the other species. Although all six species will attack man, *ochraceum* alone prefers man to other animals when given the choice of both side by side (Table II). Because of its relative abundance, *metallicum* is the predominant species collected from man. All six species have been found naturally infected with filarial larvae, but in the studies of Gibson

TABLE II—Biting Preferences of Principal Anthropophilic Species of *Simulium*—Comparison between Man and other Animal Hosts. The biting preference in each group is expressed as the percentage of the total number of flies biting man and the other animal subjects. Each pair is comprised of two 6-hour observation periods. "Total number biting" represents the number of flies that were collected from both subjects during the two 6-hour observation periods.

Subject	Per cent Biting			Subject	Per cent Biting		
	ochraceum	metallicum	callidum		ochraceum	metallicum	callidum
Man	85	10	8	Man	78	61	40
Horse	15	90	92	Goat	22	39	60
Total number biting	404	985	114	Total number biting	303	259	78
Man	99	26	31	Man	99	98	100
Mule	1	74	69	Cat	1	2	0
Total number biting	670	1,183	129	Total number biting	692	333	16
Man	100	15	44	Man	99	94	100
Donkey	0	85	56	Pigeon	1	6	0
Total number biting	205	772	34	Total number biting	231	51	3
Man	98	15	41	Man	100	80	100
Cow	2	85	59	Duck	0	20	0
Total number biting	812	1,033	27	Total number biting	77	135	4
Man	99	87	92	Man	99	96	94
Pig	1	13	8	Turkey	1	4	6
Total number biting	870	255	25	Total number biting	1,077	424	33
Man	94	67	53	Man	99	94	100
Sheep	6	33	47	Chicken	1	6	0
Total number biting	2,531	902	153	Total number biting	363	88	23
Man	90	54	69				
Dog	10	46	31				
Total number biting	1,128	687	80				

From: Dalmat, 1954.

and Dalmat (1952), it was surmised that the *Onchocerca* larvae recovered from *exiguum* and *haematopotum* were probably of bovine origin. In Guatemala, the cattle and horses in the onchocerciasis zone are commonly infected with *Onchocerca gutturosa* and *reticulata*, and to date, the larval forms in the flies cannot be distinguished from those of *O. volvulus*. Unfortunately, the same species of flies bite man and domestic animals and will feed alternately on any of their hosts. We could infect all six species experimentally and have the parasite develop within them. It was difficult to keep *S. ochraceum* alive in a laboratory environment, while *veracruzianum* appeared to be the most adaptable to such conditions. It would appear from the data on host preferences, that *S. ochraceum* and *S. metallicum* are the principal culprits in the transmission of onchocerciasis in Guatemala. However, in some regions of the western onchocerciasis zone, *S. ochraceum* and *metallicum* were not present and appeared to be replaced by *veracruzianum* as the dominant anthropophilic species. Under such conditions, the latter species is undoubtedly the vector.

### BIOLOGY AND CONTROL

For the sake of brevity, and from the standpoint of major importance, the discussion that follows concerns primarily the biology and control of *S. ochraceum* and *S. metallicum*.

## A. LIFE HISTORY

1. *Simulium* (S.) *ochraceum* WALKER

The life history of this species was particularly difficult to study because of the characteristic habitat of the immature stages and the mode of oviposition. It is found breeding principally in *infant* and *young* streams which flow through exceptionally "rugged" terrain. These streams are usually concealed by a dense canopy composed of three to four layers of vegetation—emergent vegetation, overgrowth of grasses and other plants preferring a moist environment, shrubs, and low trees, and finally tall trees. Thus, the breeding places are difficult to find and somewhat inaccessible. The species is most commonly collected in streams at altitudes from 3,000 to 5,000 feet. The *infant* stream (Fig. 2) is one formed by convergence of several minute trickles of water, generally originating as collections of underground or cliff seepage. The stream may vary in width from one inch to about one foot. It has no definite wall or cross section and the water channel seems almost haphazard. The stream bed is hardly distinguishable from the contiguous dry area. Vegetation, rather than being of a truly aquatic type, appears to be composed of trailing parts of plants that grow along the sides of the water course, as well as of debris and decaying leaves that also cover the adjacent ground. Such streams may enter and emerge from the ground several times as they pass along a slope. The *young* stream (Fig. 3) in which *ochraceum* also develops, is relatively narrow, with steep walls, and V-shaped cross section. It has few, if any, tributaries and these are very short. The stream presents a zigzag, ungraded pattern, often with rapids and small falls, and sometimes with pools. Characteristically, it has abundant emergent and cover vegetation and small deposits of debris. The bed of the young stream consists mainly of an arenaceous mixture topped with small to large stones, and rarely with large rocks. In some streams, the sand accumulates around the large rock outcroppings to such a height that the upper faces of the rocks themselves form the main part of the stream bed, the spaces between them being filled with sand and gravel. The walls of the stream may be composed of earth, vegetation, rocks, or any combination of these. The streams just described may vary from a few inches in width to a few feet, the depth rarely over 5 inches. The optimum temperature for *ochraceum* breeding is between 18 and 20°C. The preferred velocity is from 1 to 10 inches per second, with a volume flow of 1 to 10 gallons per second. The optimum pH is between 7.1 and 7.5. Eggs, larvae, and pupae have been found on parts of plants floating on the surface of the streams or emergent from them. They were *Axonopus compressus* (Sw.) Beauv., *Tradescantia commelinoides* R. and S., *Tripogandra cumanaensis* (Kunth) Woodson, *Ipomoea* sp., and *Hyptis sinuata* Pohl. Occasionally, the immature stages were collected from debris vegetation, but never from hirsute plants.

The adult flies mate soon after emergence, the development of the eggs depending on the ability of the female to secure blood meals before oviposition. *S. ochraceum*, unlike *metallicum*, oviposits, usually between 12:00 noon and 2:00 p.m., by hovering above the less turbulent parts of the stream and dropping relatively few eggs in any one place on the floating emergent vegetation. It deposits three to four eggs in approximately 4 seconds. Since so few eggs are laid in any one place, it is very difficult to find them unless the female is found in the process of ovipositing. It was not realized for a long time that the hovering female was ovipositing, since all other species observed either entered the water to deposit eggs, or approached or landed on a rock or a floating leaf. Within 3 to 10 days the young larvae emerge and soon migrate to an area where the current is somewhat more forceful. The larvae pass through four stadia, and probably a fifth, in 7 to 15 days. The more mature larvae maintain themselves in the swifter currents, but just prior to pupation, they migrate to quieter sections of the stream, often on the underside of the leaves or in the shielded parts of stones where they are afforded more protection. Here the larvae spin the cocoons in which they pupate, the process taking about 5 hours. The adult emerges in from 4 to 6 days.

2. *Simulium* (S.) *metallicum* BELLARDI

This species is more adaptable to different types of breeding habitats than is *ochraceum*. Larvae are found in *infant*, *young*, *adolescent*, and to some extent in *mature* streams and are often found breeding in temporary streams of only a few weeks duration. The breeding sites range in altitude from 350 feet to over 9,000 feet and are either open to the sun or well shaded by trees and shrubs. I have already described the *infant* and *young* streams in discussing the breeding of *ochraceum*. The *adolescent* stream (Fig. 4) has the walls less steep





Fig. 1. Aerial view of the principal onchocerciasis zone along the Pacific versant of the Sierra Madre. Fig. 2. An infant stream in which *S. ochraceum* prefers to breed. Fig. 3. Young stream with definite course and steep grade, and covered by heavy vegetation.

than the young ones, the falls and rapids usually are replaced by a more graded river bed, the zigzag pattern gives way to meanders, and the river assumes a dendritic pattern rather



than a singular one. The bed is approximately like that of the young stream, still supporting a large growth of emergent vegetation and being shaded by dense growth. The more *mature* stream exhibits a broad U-shaped profile (Fig. 5). There is usually a complex network of streams with piracy as a common manifestation. Because of the extensive number of tributaries, a large part of the adjoining region is brought more to the slope of the river bed.



Fig. 4. Adolescent stream of the onchorcerciasis region. Fig. 5. Mature stream, showing formation of sandbars. Note how the region adjacent to the stream has been brought to the slope of the stream bed. Fig. 6. Man-made water channel (toma) that conducts water to the plantation area from a nearby stream. These channels serve as good breeding grounds for *S. metallicum* and other species of black flies. Fig. 7. Determination of host preference of simuliid flies. Collections made from man and cow simultaneously.

Deposition may occur, forming narrow flood plains, sandbars and beaches. In the *adolescent* stream, good numbers of *metallicum* can be found, but relatively few in the mature ones where the zoophilic species abound. *Metallicum* will also breed in the "toma" of a plantation (Fig. 6) which is a man-made water channel used to lead water from the natural source to the area of greatest need on the plantation. The optimum stream conditions for *metallicum* breeding are: Width, 1 to 8 feet; depth, less than 1 foot; temperature, 17 to 20° C; velocity, 8 to 20 inches per second; volume flow, 1 to 10 gallons per second but with great tolerance above this; pH, 6.6 to 8.0. Eggs, larvae, and pupae have been found on the plants serving as a substrate for *ochraceum*, as well as on a number of additional ones. One of these, *Renealmia* sp., is the favorite of several of the zoophilic species. Like with *ochraceum*, the adults mate very soon after emergence, the females requiring a blood meal for the eggs to develop. If the current is very rapid, the female approaches an appropriate leaf at the surface of the water and deposits an egg without apparently landing; it then hovers above the leaf and returns to the same spot to deposit the second egg. The eggs are laid one in



2 seconds, contiguous to each other, but forming no general pattern and never overlapping. Should the stream be relatively slow moving, the fly will actually land on the leaf to deposit its eggs. Often several females, at times as many as 30, have been seen ovipositing on a single leaf of *Renealmia* sp. One fly can deposit from 150 to 500 eggs in a mass and seems to prefer the hours from 5 p.m. to 6 p.m. for oviposition. The first-stage larvae emerge in from 3 to 20 days and soon arrange themselves in the smaller currents, most frequently attaching to leaves of plants, but at times to stones and rocks. The larval development follows that of *ochraceum*, with pupation occurring after 6 to 20 days. The cocoon is woven on leaves or stones in a manner similar to that of *ochraceum*. If the case is on a flat surface, it bears winglike lateral extensions on each side; if on rocks, inserted next to the vein on the underside of a leaf, or on a round twig, it may lack the extensions. The adult emerges in from 4 to 10 days.

## B. BIOLOGY

In the course of working with *S. ochraceum* and *metallicum*, several interesting aspects of their life and their relations to humans were observed.

### 1. HOST PREFERENCES

To determine the host preferences of these species, two types of experiments were performed. In one, several groups of men, composed of two individuals each, were assigned to areas where black flies were abundant. One individual served as a subject, removing his footgear and clothing from the waist up and rolling up his trouser legs. The other person collected specimens as they began to bite the subject. In the second series, flies were collected from both a human and other animal subject, these situated next to each other (Fig. 7). From the fact that 65% of the black flies collected from human subjects were *metallicum*, it might be assumed that this species is more anthropophilic in its feeding habits than *ochraceum*; however, the reverse is actually true, *metallicum* merely being the numerically dominant species (Table II).

### 2. BODY REGIONS PREFERRED

It will be seen in Table III that when a person is fully exposed to the bites of both species, *ochraceum* shows definite preference for the upper regions of the body, while *metallicum* prefers the lower regions. However, when the preferred region is covered, either one of the species will bite any part of the body that is exposed. Actually workers on the coffee plantations are exposed to all three species. The men usually roll up their trousers and shirt sleeves, while the type of clothing worn by the women permits flies to bite on the head and neck, as well as on the lower limbs. Since it is usually stated that the upper regions of the body, preferred by *ochraceum*, contain higher concentrations of microfilariae in the subcutaneous tissues, numbers of each species were fed on the upper torso as well as on the legs. It was found that the flies fed on the thigh of an infected individual will take up at least as many microfilariae as those fed on the upper regions.

### 3. FEEDING TIME

In the Yepocapa onchocerciasis zone, Gibson (1951) reported that only 0.38 percent of wild-caught *ochraceum*, 1.04 percent of *metallicum*, and 0.62 percent of *callidum* were naturally infected with *Onchocerca* larvae in all stages of development. Because of this low rate, and obviously still lower rate with infective larvae, these often being found in the thoracic region rather than in the head, some workers have doubted that *Simulium* flies could alone be the vectors of onchocerciasis when compared to what occurs in mosquitoes, *Culicoides* spp., and other insect vectors of filariid worms. Actually, since the over-all natural-infection rate in *Simulium* spp. is so low, it should be expected that the great majority of infected flies would contain developmental stages of the filariid larvae rather than infective forms. A study of the feeding time of these species offers a plausible explanation for the paucity of infective larvae in the head region of wild-caught flies.

The time required for several hundred of each of the three most important species to feed (not necessarily to engorge completely) was observed over a long period of time, using various subjects. In Table IV, it can be seen that the mean feeding time for *ochraceum* was 5.1 minutes, for *metallicum* 3.8 minutes, and for *callidum* 4.4 minutes. The differences can be partially explained by the nervousness of *metallicum* and *callidum*, which are more easily dislodged by movement or shadows crossing their path. Because of the relatively long



feeding periods of these flies, there is sufficient time during a meal for migration of larvae from the thoracic region to the mouth-parts where they can be introduced into the host.

#### 4. EFFECTS OF ENVIRONMENTAL FACTORS

To determine the relation of time of day, air temperature, relative humidity, and light intensity to the feeding habits of *S. ochraceum*, *metallicum*, and *callidum*, a series of specially designed experiments was carried out. On 95 days, from the hours of 6:00 to 6:30 a.m. until 5:30 p.m., subjects were exposed in the field to the bites of flies, each subject being accompanied by three observers. In some of the experiments, the subject was rotated with the movement of the sun, while in others he remained stationary. Every ten minutes, the time, temperature, reflected light from the chest and back of the subject, general weather conditions, species of flies biting, part of body attacked, and whether the fly was biting in an area exposed to the sun or shade were recorded. The relative humidity was taken at half-hour intervals only.

TABLE IV—Feeding Time of the Three Principal Anthropophilic Species of Simuliidae, Expressed as Mean Feeding Time of Flies Biting on Preferred Parts of Body of Human Subjects\* (*S. ochraceum* biting on upper torso, *S. metallicum* and *S. callidum* biting on lower limbs).

Species	Number of flies	Mean (minutes)	Range (minutes)	Standard deviation	Standard error of mean	Standard error of difference		
						Species involved	Standard error	Validity
<i>ochraceum</i>	782	5.1	1-19	2.66	0.10	<i>ochraceum-metallicum</i>	$1.3 \pm 0.15$	Valid
<i>metallicum</i>	480	3.8	1-19	2.53	0.11	<i>metallicum-callidum</i>	$0.6 \pm 0.19$	Valid
<i>callidum</i>	197	4.4	1-14	2.2	0.15	<i>ochraceum-callidum</i>	$0.7 \pm 0.18$	Valid

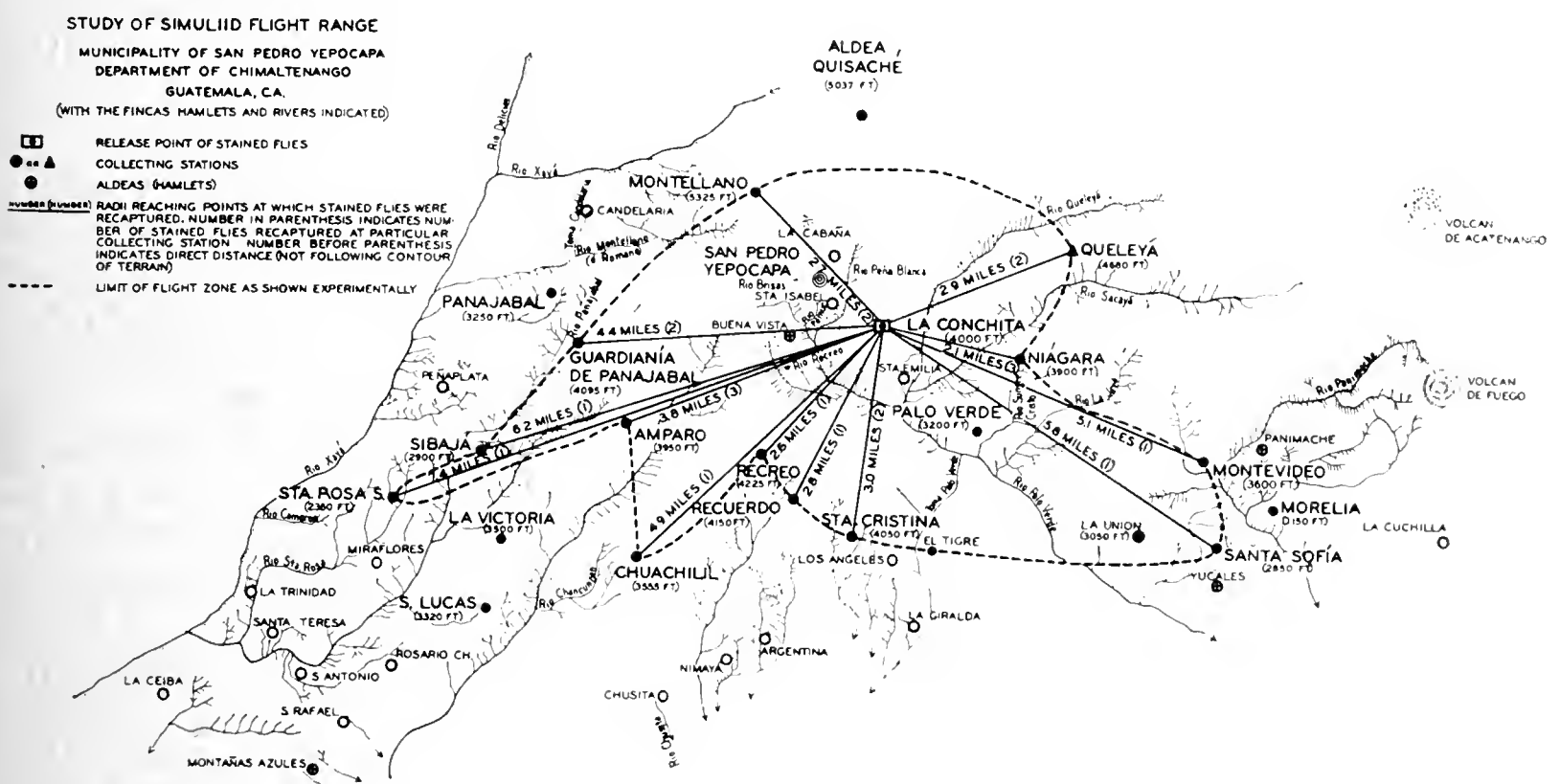
\*The validity of the difference of mean feeding time of these species is expressed by the standard error of the difference of the means.

It was found that *ochraceum* bites from 7:00 a.m. until 4:00 p.m., the most active feeding being from 8:00 to 10:00 a.m. Although the optimum hours of feeding of *metallicum* coincide with those of *ochraceum*, it continues to feed in good numbers until 5:30 p.m., *S. callidum* differs from both of these species in that it prefers to bite from dawn to about 9:00 a.m. and again from 3:00 or 4:00 p.m. until twilight with very little activity during the middle of the day. *S. ochraceum* feeds in the sun at air temperatures from 15 to 40° C, with its optimum at 34 to 35° C. In the shade, it will start feeding at slightly lower temperatures. *S. metallicum* starts actively feeding at about 22° C in the sun and at 17° C in the shade and then continues to feed with almost equal avidity until 40° C. At high relative humidity, *ochraceum* is more active than at lower humidity, its optimum being 81 to 90 percent. *S. metallicum* seems to bite about as actively in lower relative humidities as in high, although it does have a similar optimum to that of *ochraceum*. Within its optimum range, *ochraceum* prefers biting in the sun, while below that it prefers the shade. *S. metallicum* prefers shaded parts except at relative humidities below 40 percent when it prefers areas exposed to the sun. At relative humidities between 81 to 100 percent, *ochraceum* seems to bite at considerably higher temperatures than *metallicum* or *callidum*.

It was found that the three principal anthropophilic species preferred biting when the light reflected from subjects (taken with a General Electric exposure meter) was between 4 and 10 foot-candles. The intensity of reflected light, as would be expected, is related to the time of day, the clarity of the sky, quantity of vegetation giving shade, and the color of the subject's skin. Light-skinned subjects reflected much more light, at times three or four times more, than did dark-skinned subjects. In the early morning and in the evening, when the light readings were zero, few if any flies would bite the dark individuals, while they would readily bite the fairer-skinned ones. However, during the middle of the day, when the light intensity increased, there was little difference between the number of bites received by the light- and dark-skinned subjects. In open areas with clear bright sun, the light intensity is often such that only dark-skinned individuals are bitten.

It has often been said that *Simulium* species will not bite indoors, supposedly owing to inadequate light. However, *S. ochraceum* frequently, and *S. metallicum* on occasion, have been found biting inside of our laboratory during the night at light intensities that were greatly reduced. The lighting in the room where fly biting occurred consisted of two 20-watt fluorescent bulbs which were ample to give a reading of light, reflected from a fair-skinned subject, of 2 footcandles.

In discussing the epidemiological role of black flies in relation to onchocerciasis, the following three important problems must be considered: First, whether the flight range of the anthropophilic species is sufficient to permit the introduction and subsequent establishment of vector species into areas supposedly free of onchocerciasis. Second, the length of life of the females should be known in order to correlate it with the first point, thereby giving additional weight to the possibility that the disease may extend beyond its present boundaries. Third, it must be determined whether or not infection with the larval stages of *Onchocerca volvulus* adversely affects the flight range and longevity of the flies. If infection greatly reduces flight range and life span, it may partially explain the restriction of the disease zone to its presently known limited confines. From the standpoint of insecticide control of the anthropophilic species, the first two considerations would indicate to what extent infiltration by the flies from nontreated to treated areas might be expected. This information would serve in establishing the size of the area that must be treated in order to achieve adequate control.



In one flight range study (Dalmat, 1950), 19,580 stained flies were released on a single day, of which 21 were recovered at distances from 2.1 to 7.4 miles (Fig. 8). In the second study (Dalmat, 1952), of 66,544 flies released 31 were recovered from 1.0 to 9.7 miles from the release point. These distances do not necessarily represent the actual flight. Considering the extreme irregularities of the terrain, some of the ravines traversed being over 500 feet deep and a mile across, it is quite probable that flies landed several times and that the distances noted actually should be much greater. One stained *metallicum* was recovered 3.8 miles from the release point within one day. This suggested a very rapid flight and the likelihood that the flies travel great distances.

To determine longevity, 40,083 flies were stained over 12 days, a different dye being employed each two-day period to make possible the tracing of recaptured flies with an error no greater than one day (Dalmat, 1952). Collecting stations were not fixed, being established closer to the release point at the beginning of the study and more distant from it toward the end. Ninety-one stained flies were recaptured. From Table V, it can be seen that the greatest longevity recorded was 85 days, which was for *metallicum*. Since there were continuous collections up to that time, this capture cannot be disregarded as atypical. Obviously, the findings represent only an approximation of the natural longevity since it is not known how long these wild flies had been living prior to staining, or how much longer they would have survived had they not been killed for examination after recapture.

TABLE V—Longevity of Anthropophilic Simuliids.

Longevity (days)	<i>Simulium</i> (S.) <i>metallicum</i>	<i>Simulium</i> (S.) <i>ochraceum</i>	<i>Simulium</i> (L.) <i>callidum</i>
3	2	—	2
5	1	12	—
7	9	5	1
9	1	—	—
10	3	1	1
11	7	—	—
12	—	1	—
13	1	1	—
14	—	2	—
17	1	—	—
20	—	—	1
21	1	2	—
24	1	—	—
25	16	4	—
26	2	—	—
27	1	1	—
38	1	—	—
39	1	—	—
41	3	—	—
52	1	—	—
64	1	—	—
66	1	—	—
68	1	—	—
72	2	—	—
75	1	—	—
77	1	—	—
85	1	—	—
Totals	60	29	5

To determine the effect of infection on the flight range and longevity (Dalmat and Gibson, 1952), 40,474 wild flies, prior to being stained and released, were infected by permitting them to feed on six onchocercotics previously proven to be very infectious to flies. Sectioning of the 42 recaptured stained flies revealed only three infections and the developmental stages found left little doubt that the flies had become infected by the experimental feeding rather than by casual feeding either before or after release. The maximum flight of these infected flies was 2.9 miles and the longest flight recorded was 3 to 4 miles. Some of the noninfected flies recovered had flown as far as 9.6 miles.



From past experience, it was known that approximately one-half of the flies fed on a heavily-infected person ingest microfilariae and subsequently become infected. On this basis, if no mortality of infected flies occurred, infection should have been found in 21 of the 42 recaptured flies. Since only three infected flies were recovered, the possibility is suggested that infection with *O. volvulus* has a deleterious effect on the flies and causes the early death of many. The relatively short distances covered by the infected flies also suggests an effect of the infection. Lebied (1950), in his studies of the development of *O. volvulus* in *Simulium damnosum*, concludes that pathological changes caused by "sausage" forms developing in the fibers of the indirect flight muscles presumably restrict the flight of infected flies, thereby limiting the spread of onchocerciasis.

From the above data, it is obvious that in any control program aimed at the reduction of the anthropophilic species, whether infected or not, relatively large areas would have to be included to minimize infiltration of flies from outside of the treated region. If the program were to be directed solely against infected flies, with a view toward preventing their migration to neighboring noninfected regions, it would not have to be as extensive, although it would have to include an area at least 2.9 miles beyond the infected region. In any program for the control of human onchocerciasis in Guatemala, it would seem advisable to attack both the infected and noninfected flies.

#### 6. RESTING PLACES AND HEIGHT RANGE

Before considering control of adult black flies, it was of prime importance to learn on what surfaces they alight. Much money and effort may be spent uselessly by indiscriminate use of insecticide. It was found that as the sun set, the flies migrate downward and that at nightfall, the flies actually worked their way down to the bases of plant stems close to the ground level or, at times, slightly beneath the surface.

During the day, flies not only were found on leaves of vegetation close to the ground level, but also in the trees up to 50 feet where they were resting on leaves or branches and as high as 120 feet where they were captured while biting.

#### 7. SEASONAL FLUCTUATION IN POPULATIONS

Larvae, pupae, and adults of *S. ochraceum* are found in any one region throughout the year. However, there are two peaks of larval production, one in April and another in October, which occur, as might be expected, at the time when the adult populations are at a minimum (Fig. 9). Since *ochraceum* breeding areas are principally the minute trickles (*infant*) and young streams, the larvae are very much affected by the torrential rains during the rainy season. The flash flooding of these breeding areas dislodge myriad larvae and do much to keep the fly population in check. From the middle to the end of the dry season, the larval population also declines due to drying out of many of the smaller streams. *S. metallicum* does not have such marked peaks as *ochraceum* principally because its breeding places include larger, more stable streams.

### C. CONTROL

Along the Congo River in Leopoldville, Belgian Congo, where larval control was impractical because of the tremendous quantities of insecticide that would have to be employed, aircraft spraying of the marginal vegetation and soil was practiced (Wanson, Courtois, and Lebied, 1949). In this case control was excellent since aircraft could approach close to the area to be treated, and since the vector, *S. damnosum*, was breeding principally in the main parts of the single river and not in minute tributaries that are widely dispersed and protected. The adults also did not seem to migrate far from their breeding places. Because of the extensive flight range and great longevity of the adult *S. ochraceum* and *metallicum* in Guatemala, and the dispersion of the breeding regions, control of the adults would entail treatment of widespread areas that make such control impractical without the use of aircraft. Because of the extremely broken character of the terrain in the Guatemalan onchocerciasis zone, the location along the slopes of volcanoes that make flying treacherous, and the dense canopy of vegetation screening the underbrush and ground from the penetration of sprays, aircraft control of adults could not be used. In attempts at using portable ground machinery in the area, a Microsol Mist Generator "304" (Silver Creek Precision Corp.) was mounted on a horse that was led through the coffee plantations. Even in these more cleared areas, headway was slow, requiring constant removal of vines, underbrush,

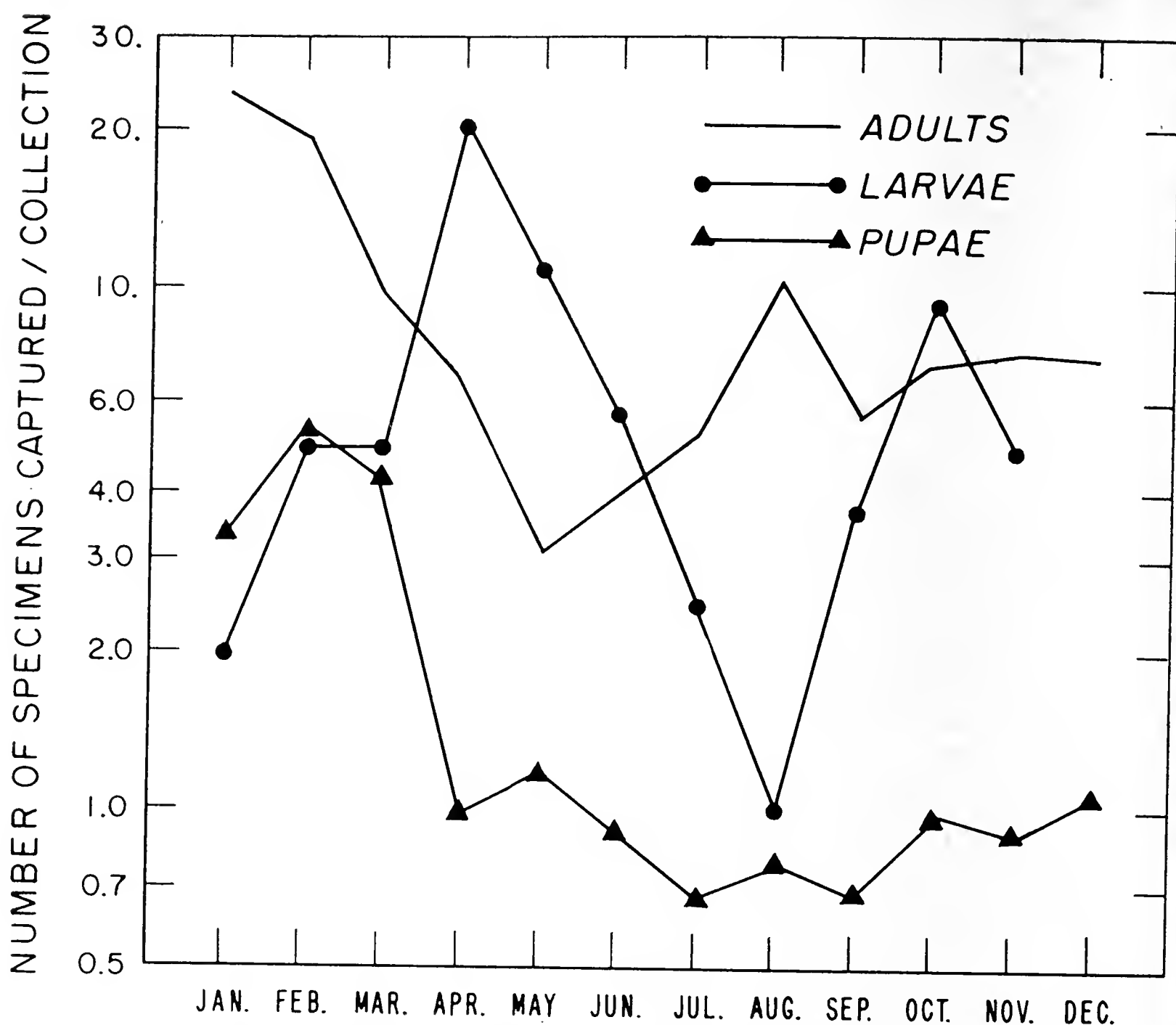


Fig. 9. Fluctuation in population of *S. ochraceum* throughout a year period, expressed as the average number of specimens captured per collection during month periods. (Prepared on 3-cycle semilogarithmic paper).

and extending branches of trees, and it was difficult to reach the upper layers of vegetation. To treat large enough areas within an appropriate time interval for adequate control, there would have to be too large an expenditure for equipment and personnel. Where mist treatment was carried out in small open areas, no reduction in adults was noted the day after application.

It was early decided that larval control was the only solution to the problem in Guatemala. Area larval control of black flies was preceded by laboratory investigations which led to the development of a technique for screening chemicals as possible larvicides, and the testing of several hundreds of such compounds (Lea and Dalmat, 1954). This was followed by preliminary field tests with various commercial insecticides applied as larvicides to determine their effectiveness in different types of streams (Lea and Dalmat, 1955). Then a small-scale program was carried out to study the problems involved in reducing the vector population by larviciding all streams within a given area. Although EPN and heptachlor gave better results than DDT in streams of less than 500 gallons per minute, we concluded, as did Hocking (1950) in Canada, that DDT was the most satisfactory general insecticide to use in the final pilot study of area larval control. Since DDT applied as wettable powder gave very poor results in the small, slow rivulets, it was decided to use the emulsion form.

The region of Yepocapa where our work was carried out contains a vast network of rivulet-fed streams. In an area of approximately 75 square miles, there were over 1,500 small streams and rivulets during the dry season. The greater proportion of the small rivulets were located at the higher altitudes and as these flowed down the mountainsides, they joined others to form slightly larger streams until toward the lower limits of the zone, fairly good-sized *adolescent* and *mature* streams were formed. More than 85 percent of all streams were less than 200 feet long, and morphologically of the *infant* and *young* type

which harbor all of the *ochraceum*, and a good part of the *metallicum* breeding. The greatest number of these rivulets had volumes less than 100 gallons while the young streams might go up to 1,000 gallons per minute. In the small streams with volumes from 10 to 120 gallons per minute, it was difficult to calculate the rate of flow by timing a floating object over a measured course or by using a pygmy current meter, methods generally used in the larger streams. However, the work did require a simple, rapid, and portable means of making numerous volume determinations in the field. For this, a small metal and canvas canal (Lea, 1955) was employed which had been calibrated previously to indicate directly the flow in gallons per minute, when a stream was channeled through it.

The area-wide larviciding was carried out between December 1, 1952 and May 15, 1953. It was primarily a pilot study to determine the problems which might be encountered in future large-scale attempts to control the vectors. It was realized that the time available for the study would not permit the work to be carried on long enough or to be extended over a sufficiently large area to effect any significant reduction of the adult population. However, even during the short period the project was operating, sufficient information was obtained so that methods and plans could be suggested for a future control campaign.

We already had certain information concerning the treatment of different kinds of streams from our earlier field studies (Lea and Dalmat, 1955). We had established that DDT emulsion would control larvae effectively when used at 1 ppm for 60 minutes or 30 minutes. Subsequent tests using 0.1 ppm for 30 and 15 minutes produced similar results. A further cut to 0.1 ppm for 3 minutes still gave as effective control in short rivulets as treatments of greater duration. Larvae were eliminated for a distance of at least 100 feet in rivulets of 20 gallons per minute and for 200 feet at 40 gallons per minute flow. It was encouraging to find that a treatment of such short duration was so effective in the small rivulets, for it would mean a considerable reduction in the number of man-hours spent in larviciding the hundreds of short rivulets which had made larval control in this area seem almost impossible. This concentration was then tried in somewhat larger streams, where it was found that, up to a certain limit, the greater the volume of the stream treated at 0.1 ppm for 3 minutes, the greater the distance of kill. However, in streams of volumes greater than 4,500 gallons per minute, the application of 0.1 ppm for 3 minutes did not produce the same proportionate increase in distance of kill. In the larger streams, 2 ppm for 3 minutes was somewhat less effective than 0.1 ppm for 60 minutes, although the same quantity of insecticide was involved. The latter concentration applied to a river of 90,000 gallons per minute flow completely cleared it of larvae for at least 20–25 miles. No doubt the distance of kill is largely controlled by the rate of dilution of the larvicide with water from tributaries, as well as the rate of "settling out" of the insecticide. With regard to the latter condition, at least two factors appear to be important: (1) current speed and (2) turbulence of the water. The small rivulets were so sluggish that the water's surface was rarely broken. The larger streams, on the other hand, were swift and contained numerous rocks and boulders which created small falls and ripples. This churning produced greater mixing action, and thereby prolonged the time during which the emulsion would remain in suspension. Certainly the results of experiments in swift mountain streams cannot be taken as a true indication of the effectiveness in the more sluggish streams of the coastal plain, any more than test data from one country can be expected to reflect exactly the outcome in another, but the principles and techniques in one area may be useful elsewhere.

As the rivulets were generally not over 200 feet in length, one application at their source was usually sufficient to rid them of larvae. The longer streams were treated at several points along their course with 0.1 ppm DDT for 3 minutes, the distance between treatments depending on the distance of kill attainable with this concentration and on the particular stream-volume as calculated at the previous point of treatment. Since each main stream was followed downstream in order to treat all its tributaries, even those with volumes greater than 5,000 gallons per minute could advantageously be given repeated treatments of 0.1 ppm for three minutes, although each treatment would not be effective for more than 2 miles from the point of treatment. However, in the longer streams and in places where inaccessibility hindered such frequent applications, either 0.1 ppm DDT for 60 minutes or 2 ppm for 3 minutes were used effectively since the distance of kill was greater.

The field personnel were always sent out in pairs, each pair assigned to treat the tributaries of one or two of the major stream systems. The work was begun at the headwaters of each main stream and each of the tributaries, along with its branches, was followed to its source for treatment. In a long rivulet which required interval treatments and which had branches joining it, the volume at each point of treatment could be satisfactorily estimated by taking the sum of the volumes of all the upstream branches which entered it. Each pair marked the number and volume of every tributary on a convenient rock or log near its entrance to the main stream, at the same time indicating these on a rough schematic map. For the sake of ease in dispensing the DDT emulsion, it was prepared in 4 c.c. vials with either black or red screw tops. Knowing that a half-full black-topped vial supplied sufficient concentrate to treat a rivulet with a volume of 10 gallons per minute, and that the contents of a half-full red-topped vial was adequate for a 50 gallons-per-minute stream, the operators could treat any of the small breeding streams in the area by applying the contents of an appropriate combination of one-half, three-fourths, or completely full vials. The necessary volume of DDT concentrate was emptied into a small can and then diluted with stream water to a known level that permitted the resulting emulsion to be dispensed through a rubber-tube siphon in 3 minutes.

Since it was necessary to mark tributaries and calculate their volumes only at the time of the first application, subsequent treatments could be made in a much shorter period of time. Thus, it was possible to increase the number of streams treated by each pair, thereby gradually expanding the total area under control. Because of the continuous breeding of the black flies throughout the year, their relatively short life cycle and their extensive flight range, 3 weeks after the first application streams would be reinfested and require retreatment.

At the beginning of the larviciding program, checks on larval populations in treated streams were made frequently, but after it was demonstrated that larviciding was effective in eliminating the larvae, only occasional spot checks were made. Master maps of each main stream system were prepared from the rough schematic maps made by the field personnel and these served for orientation in subsequent larviciding of the same streams.

During the period of control and for three additional months, 8 adult collecting stations were maintained to sample adult black-fly populations and to note fluctuations due to larviciding. Stations outside of the experimental area served as controls.

Although larviciding was carried out for a relatively short period, and over a comparatively small area, it was demonstrated that streams could be kept clear of the larvae and that the adult biting population was depressed in spite of certain infiltration from outside of the treated area.

We estimated that the costs of a control program in an area of 100 square miles in the Guatemalan onchocerciasis zone would be \$800.00 per month if no help was contributed by the local plantation owners, and only \$480.00 if part of the labor was to be furnished by them. Since the larval population of the principal vector, *S. ochraceum*, drops off significantly during the rainy season and toward the end of the dry season, it would probably suffice to treat the streams at 25 to 30-day intervals (the approximate life cycle) during the early part of the dry season, November through February, and at 1½ to 2-month intervals during the rest of the year. It seems perfectly feasible to me for Guatemala to carry out a campaign against the black flies, at least in its principal onchocerciasis zone of 500 square miles. If this were combined with drug treatment of infected individuals, infection in humans and in the flies would probably reach such a low rate that transmission would be negligible.

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## DISCUSSION

R. M. GORDON. Is it correct that the developmental forms in *Simulium* derived from equine and bovine sources are indistinguishable from those derived from human cases of *O. volvulus*?

H. DALMAT. That is true. We are unable to distinguish in the flies the developmental forms that are derived from humans, horses, or cattle. This, of course, complicated data on natural infection studies in wild-caught flies.

R. LEVI-CASTILLO. It would be interesting to know which are the natural reservoirs of onchocercosis in Guatemala.

H. DALMAT. The only animals we have found infected with microfilariae in nature are the cattle and horses and these definitely are not *O. volvulus*. It thus appears man is the only reservoir.

L. DAVIES. Has Dr. Dalmat any information on whether the infection rate with *Onchocerca* of his *Simulium* species varies in any regular way during the day?

H. DALMAT. No such study has been made. However, due to the low infection rate in the *Simulium* adults and diurnal variations in the population of each of the species involved, I do not think this information could be easily determined.





# Control of *Simulium* Vectors of Onchocerciasis in Uganda

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## ABSTRACT

*Simulium damnosum* breeding in a 42-mile stretch of the Victoria Nile near Lake Victoria infested an area of about 3,000 square miles. Onchocerciasis is widespread in the area. *S. neavei* is the more important vector in Uganda and is found everywhere in free-flowing streams on forested mountain slopes. Methods of controlling the two species with DDT are described.

## SIMULIUM DAMNOSUM

The presence on the Victoria Nile of the pest later described from specimens collected in the same area as *Simulium damnosum* was noted by the explorer Speke in 1863 on his journey of discovery to the source of the Nile. In 1933 Gibbins and Lowenthal incriminated *S. damnosum* as the vector of cutaneous and ocular onchocerciasis occurring in African inhabitants of the Mabira Forest bordering the Western banks of the Nile near Jinja.

Surveys have shown that an area of approximately 3,000 square miles bordering a 42-mile reach of rapids on the Nile immediately below its point of origin from Lake Victoria was infested with *S. damnosum* to an extent at which the biting nuisance of the fly was responsible for progressive depopulation of agricultural holdings. Onchocerciasis is widespread throughout the area and in communities within ten miles of the river, the incidence of the disease as indicated by single skin snip biopsy is 100% of the adult population. The manifestations of the disease include intense lichenification of the skin, gross adenolymphopathy, scrotal adenolymphocoeles, and the "endemic dwarfism" postulated by Raper and Ladkin as being a sequela of onchocercal involvement of the pituitary in young children. Ocular involvement occurs but no figures are available for the incidence of blindness attributable to onchocerciasis. In 1947 the decision to establish a large hydro-electric power station on the Owen Falls of the Victoria Nile stimulated enquiries into the possibilities of controlling the vector simuliid in the interest of the health of construction workers.

Surveys showed that breeding of *S. damnosum* was confined to a 42-mile stretch of rapids in the main stream of the Nile and that none of the tributaries (which are little more than storm water erosion gulleys) were infested.

The work of Wanson *et al.* in freeing the Stanley pool rapids on the Congo River of *S. damnosum* with DDT applied to the marginal vegetation as a residual adulticide applied from aircraft was first considered as a basis for control work on the Nile, and a small-scale airspraying experiment was undertaken. DDT in a 10% oil solution was applied to the marginal vegetation at a rate of 20 mgm. per square metre in three five-yard swaths, one along each bank of a six-mile course and the third over the central islands. Eleven sorties at three-day intervals were planned. A very significant decrease in the adult population was apparent after the first application. The results were in fact considerably more complete than could have been achieved by adulticidal action over a mere six-mile fraction of a 42-mile stretch of breeding sites: a significant diminution of fly density was noticeable throughout the whole of the infested area. Investigation showed a marked larvicidal action reaching several miles downstream from the reach sprayed. This larvicidal action was the result of DDT solution which had missed its target of marginal vegetation and fallen on the water. The concentration of DDT so applied would have been of the order of 1 part DDT in 40 million parts of water maintained for 30 minutes.

The cost of aircraft operation over the whole length of the breeding sites and the very considerable flying hazards to which the pilots were exposed discouraged the adoption of air spraying and the larvicidal side effects supported the adoption of a larvicidal campaign.

The mechanical difficulties of applying a larvicide to inaccessible breeding sites of the 300-yard wide Ripon Falls cataract at the outflow of the Nile from Lake Victoria delayed the start of operations. A powerful ex-Air Force air-sea rescue launch equipped with a Coventry Climax fire-fighting pump was finally obtained. A simple plumbing modification enabled the insecticide to be aspirated directly into the volute chamber of

the pump, mixed intimately with a large volume of lake water and projected some 80 feet in the main fire fighting jet. Due to the limited carrying capacity of the launch, a formulation of the highest DDT concentration locally available was used, this being a 27% "mayonnaise" type emulsion concentrate.

On 3rd June 1952 the first of 12 weekly applications calculated to give a concentration of one part DDT in  $2\frac{1}{2}$  million parts of water for 30 minutes was made. Forty-eight hours after this application no living *Simulium* larvae could be found at any previously stocked breeding site on the entire 42-mile stretch of rapids. After the third application the adult fly count had declined from over 100 fly per boy-hour to zero. Thereafter no adult fly were captured until 16th Oct. 1952, when a single specimen appeared. Thereafter a few larvae of *S. damnosum* began to reappear on the breeding sites below the Ripon Falls and it became apparent that our insecticide had failed to spread out sufficiently to cover all the sites on the cataract. It was feared that the operation had failed and that rapid repopulation of the breeding sites would be inevitable. This fear was unrealised and practical control with an average fly count at below one fly per day to eight bait-boys was maintained for  $3\frac{1}{2}$  years. The failure of *S. damnosum* to repopulate the Nile breeding sites is attributed by the author to two factors. 1. The insecticide fundamentally disturbed the ecological balance of the river bed. Filter-feeding arthropods were generally destroyed and the diatoms and algae spores previously eliminated by these filter-feeders settled down and the previously clean surfaced rocks and trailing vegetation became rapidly covered with a dense mat of slimy vegetable growth, quite unsuitable as a substrate for *S. damnosum*. This species failed to reappear in significant numbers and was replaced by *S. adersi*, previously not noted as breeding in the Nile but common on the algae-grown wave-beaten rocks of the shores of Lake Victoria. 2. An additional factor was the introduction of sudden and frequent fluctuations in the level of the Nile waters downstream of the Owen Falls dam consequent upon experimental manipulation of the control sluices, when the hydroelectric plant was being taken into operation. These variations in water level were demonstrably sufficient both to "drown" and to dry out whole generations of *Simulium* pupae.

Despite the slow progress of repopulation it was obvious that the activity of *S. damnosum* would eventually increase to a level at which the transmission of onchocerciasis would proceed. It was accordingly planned to re-dose the river as soon as the operation of the Owen Falls dam had impounded water to Lake level and thus inundated the Ripon Falls and all intervening breeding sites. This second series of applications was started on 2nd May 1956. Ten weekly applications of a 12.5% oil solution of DDT to give a concentration of 1 part DDT per 3 million were made by gravity directly into the sluices of the Owen Falls dam and have apparently been completely successful, the fly count remaining at zero at the time of writing.<sup>1</sup> Whether the elimination of the Ripon Falls breeding sites, which were probably incompletely covered during the 1952 series, has made it possible to achieve complete eradication of *S. damnosum* from the Nile sites remains to be seen.\*

### SIMULIUM NEAVEI

The Nile focus of *S. damnosum* was the most notorious in Uganda; in fact, in 1946 there were no Departmental records of the occurrence of onchocerciasis elsewhere in Uganda than in the immediate vicinity of the Nile rapids near Jinja. Although the material from which *S. neavei* was originally described is labelled "Western Ankole, Uganda," there were likewise no official records of the occurrence of this vector in the Protectorate.

Surveys have since shown that *S. neavei* is by far the more important vector in Uganda since very few rivers exist which provide conditions suitable for *S. damnosum*, whereas a major part of Uganda is mountainous and *S. neavei* occurs wherever streams descend forested slopes and escarpments. Wherever a human population exists within about 10 miles of such slopes, onchocerciasis is commonly encountered.

There are several indications that *S. neavei* is a more efficient vector than *S. damnosum*: there are in Uganda several examples (mostly among European children) where severe clinical symptoms, including ocular complications, have occurred after remarkably short exposure to fly on the outer fringes of an infested area where the conditions postulated by Kershaw for the development of severe onchocerciasis infection transmitted by *S. damnosum* have not been satisfied.

<sup>1</sup> Still zero on Nov. 1, 1956.

Control of *S. neavei* is as yet hampered by an imperfect knowledge of the bionomics of the vector and of the freshwater crabs in association with which the early stages are exclusively found. At the moment very arduous physical surveys must be made to discover and search even the smallest infant streams flowing down trackless mountain slopes. In the absence of any real knowledge of the flight range of *S. neavei* it must be assumed that local control measures to protect a specific locality are unlikely to succeed and the whole of any continuous range of ecologically connected habitat must be treated in the course of any control operation. Such widespread operations are a severe strain on the trained manpower available and attempts are being made to develop a simple technique available as a "help yourself" community development effort. The use of DDT water-miscible oil concentrates adsorbed on Vermiculite contained in calico bags, which can be anchored in the streams, has shown considerable promise when the introduction can be made at points of very high turbulence giving a good stirring action, but have proved disappointing in the more sluggish streams. DDT in plaster blocks are useful but the blocks do not stand transport in the hands of local labour. These slow emission techniques produce an initial concentration of less than one part of DDT in 20 million parts of water and a mean concentration of the order of one part in 100 million over a 48-hr. period. We wish as far as possible to avoid the use of concentrated solutions applied over short periods of time since, unless such dosing is carried out under European supervision, a danger of overdosing and consequent killing of fish will exist. This must be avoided at all costs, since the superstitious peasantry is far from convinced that our control efforts are not designed to poison themselves and their livestock in the interests of European annexation of their land.

Despite these difficulties, the control of *S. neavei* is an organizational rather than a technical problem. Pilot schemes have shown that, given adequate manpower, complete control may be established.

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#### DISCUSSION

C. B. PHILIP. Was there any effect on fish in the 40-mile control stretch? Are fish or fish food locally important?

G. R. BARNLEY. No direct effect on any fish was observed. There is no fishing of economic value in this stretch of the Nile, but large Barbel provide sporting amenities. The indirect effect of altering the available fish food distribution must be considerable.

D. G. PETERSON. What has been the effect on *S. damnosum* of the water level fluctuations caused by operation of the dam?

G. R. BARNLEY. It is not practical to "turn off" the flow through the dam for any length of time but an accidental fluctuation of level resulting from an engineering defect dried out an entire generation of pupae and resulted in a considerable slowing up in the process of repopulation of the breeding sites.





# The Biology and Control of *Simulium damnosum* and *S. neavei* in Uganda and Kenya

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## ABSTRACT

*Onchocerciasis* is one of several filarial infections affecting man in Africa. Total blindness may result in severe cases. In 1926 it was established that *Onchocerca volvulus*, the helminth concerned, was transmitted from man to man in Africa by *Simulium damnosum*. This blood-sucking fly occurs in its immature stages in the turbulent, fast-flowing waters of streams and rivers, including major water courses like the Nile, Volta, and Congo. The larvae and pupae attach to submerged rocks, stones, and vegetation. The vector of *onchocerciasis* in hilly regions of Kenya near Lake Victoria was known by 1940 to be *S. neavei*, first described in the adult form in 1911. Although the relationship of the flies to *onchocerciasis* in Kenya was proven, the larvae and pupae evaded discovery in the hill streams for a decade. In March, 1950, they were found attached to crabs living on the river beds and not, like *S. damnosum*, on rocks and vegetation.

The film, composed from shots taken by the speaker in Kenya and Uganda in 1950, gives some account of *onchocerciasis* and its recognition in East Africa and of the biology of *S. damnosum* and *S. neavei* on, respectively, the Nile and in certain hilly districts of Kenya and Uganda. It shows turbulent stretches of the Nile now quiescent behind the Jinja Dam, scenes of the collection of crabs carrying *S. neavei* taken within a few days of the discovery of this parietic association, and the method of control of *S. neavei* with DDT which has been completely successful in parts of Kenya.

The speaker is particularly indebted to Mr. G. R. Barnley in Uganda, and Mr. J. P. McMahon in Kenya, for opportunities of photographing the scenes.

## DISCUSSION

DEANE P. FURMAN. Has a study been made of the factors responsible for the association of *Simulium neavei* to a species of crab during the larval and pupal stages of the fly?

G. R. BARNLEY. No formal study has yet been made but observations by myself and McMahon suggest that a study of crab bionomics would produce results that would enable us to neglect the dosing of large numbers of certain types of streams.



# *Simulium damnosum* in the Tonkolili Valley, Sierra Leone

By D. J. LEWIS<sup>1</sup>

British Museum (Natural History)

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## ABSTRACT

Observations were made on *Simulium damnosum* Theobald in 1955 during a short medical entomological survey of the Tonkolili Valley (Lewis, 1956b), where onchocerciasis is prevalent but blindness uncommon. Most observations were made around the village of Farangbaya which is 9° 11' north and 11° 43' west. No roads existed at the time so that all travel was on foot and there were many opportunities of observing the flies in different types of vegetation. *S. damnosum* was not very abundant so it was easy to watch individual flies biting. Particular attention was paid to the external and internal appearance of the fly in relation to physiological age, and to its biting habits.

The condition of the ovaries and oviducts nearly always served to distinguish parous from nulliparous flies, and the appearance of the abdomen, peritrophic membrane, meconium, loose pigment granules, fat-body and other structures gave useful information about the condition of captured flies. It appeared that flies which bit had been moving about at random and were not attracted to people from a distance. The proportion of nulliparous flies was about twice as high in afternoon as in morning catches, and there was a lull in activity at midday. The flies did not follow people more than a few yards, and appeared to assemble near bridges. Flies probably hesitate before landing on human skin and often hesitate before biting. Flies were numerous on cultivated land with low bushes, and comparatively scarce in evergreen forest away from the river.

Few *S. damnosum* bred in streams, and the main breeding site in the river appeared to be submerged vegetation among rapids. The species was not very abundant in the valley. The *Onchocerca* infection rate, never very high, was greater in morning than in afternoon catches, and higher where many labourers gathered than in the area generally. It was evident that the risk of infection depended largely on the number and duration of periods for which people remained stationary in certain places, particularly outside the main forest area. Regular treatment of the river with D.D.T. may prove more than sufficient to eliminate onchocercal blindness and minimize annoyance.

## THE BIOLOGY OF THE ADULT

Except where otherwise indicated the term "fly" refers to a female bred from the pupa or caught while biting or trying to bite.

### EXTERNAL APPEARANCE

In newly-emerged flies the halteres are transparent and many large cells can be seen inside them, but in many caught flies the halteres are opaque or semi-opaque owing to the presence of stored glycogen (Lewis, 1953). In some parous flies they become secondarily transparent but differ from those of young flies in that the internal cells have shrunk.

The abdomen varies greatly in size and shape and may be large and replete, or very small and club-shaped owing to the partial collapse of the second to fourth segments which are not supported by strong tergites. The abdomen is often rather large in nulliparous flies, partly owing to the meconium and fat-body and possibly a new meal of vegetable origin in the crop. In the field this enlargement sometimes indicates the presence of many nulliparous flies, and at Farangbaya led to observations on the biting times of nulliparous flies which are discussed below.

### THE PERITROPHIC MEMBRANE

When a fly sucks blood, which is necessary for the development of the eggs, a peritrophic membrane forms round the blood in the midgut (Lewis, 1953). It was thought that fragments of membrane remaining in the gut showed that a fly had sucked blood and was therefore parous. During dissections at Farangbaya, however, a small thin membrane was found in recently-emerged flies of both sexes, and it appears that such a membrane is norm-

<sup>1</sup>Scientific staff, Medical Research Council. The field work described in this paper was carried out when the writer was seconded by the Ross Institute of Tropical Hygiene to the Sierra Leone Development Company Limited.

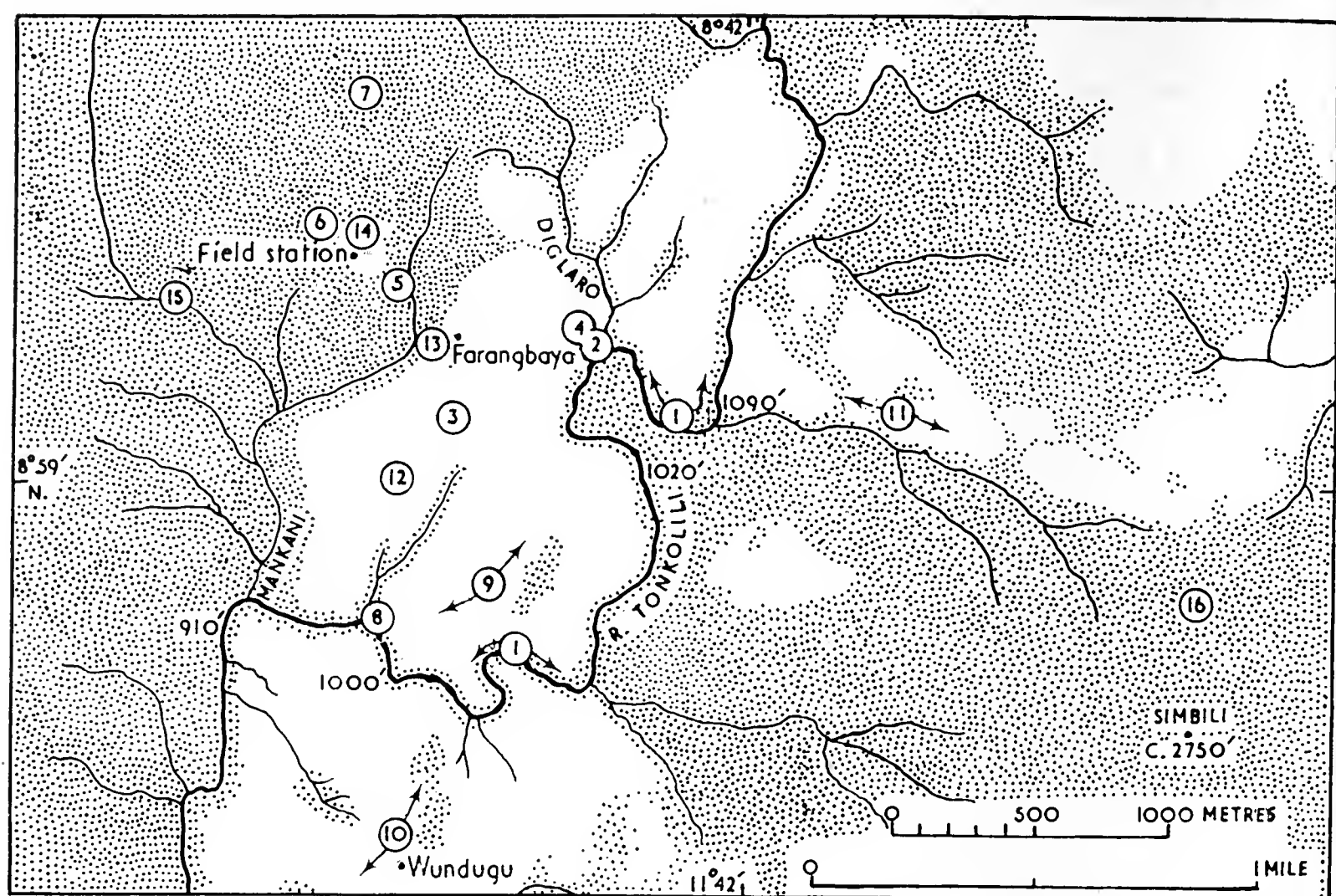


Fig. 1. The Farangbaya area showing (shaded) the approximate distribution of evergreen forest. The numbers in circles correspond to those in Fig. 3 and show where certain catches were made.

ally deposited round the meconium about the time of emergence. It follows that only thick fragments of membrane, or pieces associated with remains of blood, are evidence that a fly is parous.

Two thin membranes, one inside the other and both containing fresh blood, were seen in one fly. They had evidently been formed after the two stages of an interrupted meal. This evidence that a fly will occasionally resume an interrupted blood meal shows that xenodiagnosis by means of caught flies must be used with reserve.

The peritrophic membrane occasionally fails to develop properly in flies which have ingested many microfilariae, and allows blood corpuscles and microfilariae to pass out through the anus (Lewis, 1953). Corpuscles were seen to pass out of a fly which had gorged on a man with few or no microfilariae, so it appears that a heavy blood meal can itself delay the formation of the membrane.

#### THE MECONIUM

In a newly-emerged fly the meconium consists of two yellow bodies, derived from anterior and posterior parts of the larval midgut as one can see by dissecting pupae. The meconium soon disappears from the midgut but remnants of it are often to be seen in the hind-gut.

#### THE MALPIGHIAN TUBES

Smart (1935) found that in *S. ornatum* Meigen the large cells of the Malpighian tubes had opaque granular contents. In *S. damnosum* the contents vary and can be roughly classed as opaque, semi-opaque (with alternating opaque and clear sections) or transparent. The tubes of 57 nulliparous flies were examined in December; 50 flies had much fat-body and of these 49 had opaque and one had semi-opaque tubes; four had some fat-body and of these three had opaque and one semi-opaque tubes; three had little fat-body and of these one had opaque and two semi-opaque tubes. Of 47 parous flies examined nine had opaque tubes, 28 had semi-opaque tubes, and ten had transparent tubes. Fifteen flies with transparent tubes were examined at this season and seven were found to contain developing *Onchocerca* (one having sausage forms and six having infective forms). A high infection rate was also noted on other occasions in flies with clear tubes. Vaney (1902) found that the Malpighian tubes of *Simulium* persist throughout the pupal stage. It is presumed that

secreted material accumulates at this time and is gradually removed during the adult stage, and that clear tubes indicate that flies are old.

#### LOOSE PIGMENT GRANULES

Vaney (1902), Puri (1925), Voïnov (1928) and Epure (1937) have studied the peripheral fat-body of the larva of *Simulium* and found that, unlike that of most insects, it contains numerous brown granules. This fat-body begins to disintegrate before pupation, and Vaney traced the movements of pigmented cells from the thorax to various parts of the body. Many loose granules, probably derived from the peripheral fat-body of the larva, are to be seen when the abdomen of a newly-emerged *S. damnosum* is opened, and a few are found in flies several days old.

#### THE CENTRAL FAT-BODY

Puri (1925) did not mention a central fat-body in his description of the anatomy of the larva of *S. noller* Friederichs, but Vaney (1902), studying a French species, found a central fat-body in the abdomen of the larva, which passed unchanged into the adult stage. Davies (1955) found that abundant fat-body was invariably present in newly-emerged *S. ornatum* Meigen. All newly-emerged *S. damnosum* examined at Farangbaya were found to have much fat-body tissue and all parous flies to have little or none. It is evident that the fat-body is used before the end of the gonotrophic cycle, and that the presence of much fat-body shows that a fly is nulliparous.

Usually most nulliparous flies which come to bite have a large fat-body but some contain varying reduced amounts. The fat-body tissue was examined in 270 nulliparous flies which bit at Farangbaya, and was roughly graded according to its size. Seventy-seven per cent had much fat-body, 18 per cent some, and 5 per cent little. Several of these nulliparous flies with little fat-body, and one of those with some, had small rounded ovaries, and in one case the oocytes had shrunk.

Probably the flies with some or little fat-body had lived for several days without being able to feed on blood, and had begun to use the material stored in the fat-body although this is normally used during the first gonotrophic cycle. It is likely that flies often do fail to bite for a few days after emergence, because flies often can not bite a person who only stops for a short time, and because some nulliparous flies reach remote spots where there are few or no mammals to feed on. Rubtzov (1940) states that in Russian species the fat-body wastes considerably during starvation.

Some *S. damnosum* which fail to suck blood ingest microfilariae of *Onchocerca volvulus*. Some of these microfilariae presumably develop in the usual way, but flies with much fat-body are never found to contain developing *Onchocerca*. This may indicate that the fat-body disappears during the time taken by a microfilaria to reach the sausage stage. It is necessary to know, however, if the tissue-fluid ingested with the microfilariae can promote ovarian growth and so cause rapid disappearance of fat-body in these flies, or if it can postpone the blood meal and so allow time for the fat-body to disappear.

Some bred flies were kept under artificial conditions, in darkness at temperatures between about 23° and 27° with a supply of sugar solution and little opportunity for exercise, and were found to use up their fat-body at varying rates. After 24 hours the droplets in the fat-body of some flies were rather scattered. After three days some flies contained a reduced amount of fat-body, but after five days some still showed much of it. Two were examined after six days and one found to contain little fat-body and the other much of it with scattered globules. Probably a reduced amount of fat-body in a nulliparous fly shows that it is taking its first blood meal unusually late.

#### THE OVARIES

Wanson (1950) found that the appearance of the ovaries, but not the oviducts, was useful for distinguishing between parous and nulliparous flies. At Farangbaya (Lewis, 1956a) parous flies could nearly always be distinguished by the loose arrangement of the oocytes in the ovary and also by distention and wrinkling of the paired oviducts. In doubtful cases, due to stretching of the ovaries during dissection or to variation in the expansion of the oviducts, one could make use of one or more of the structures mentioned in preceding paragraphs.



TABLE I—Times Taken by *S. damnosum* to Appear, and Summaries of Some Catches, Between September and November.

Locality, numbered as in fig. 1	Time before appearance				Catches		
	No. of stops	Occasions when no flies appeared in 8 minutes	Waiting Time		Minutes (total)	Flies	Flies per man hour
			Total, minutes, seconds	Mean, minutes, seconds			
River (1)	76	3	265 41	3 26	345	179	55
Near Diglaro (2)	4	—	10 40	2 40	80	93	70
Bushes near river S. of Village (8)	4	—	4 29	1 7	15	8	32
Mixed vegetation S. of village (9)	161	18	490 14	3 3	230	77	20
Mixed vegetation, Wundugu area (10)	24	—	43 40	1 49	—	—	—
N.-W. of Simbili (11)	3	—	6 15	2 5	—	—	—
Grass S.-S.-W. of Village (12)*	10	2	35 40	3 34	600	284	28
Near village (13)	10	—	30 0	3 0	—	—	—
Tall grass near Diglaro (4)	1	—	4 0	4 0	60	12	12
Forest near village (5)	4	—	14 25	3 36	110	24	13
Field station (14)	6	4	38 5	6 21	240	27	7
Forest near field station (6)	1	—	1 45	1 45	60	4	4
Forest near Mankani (15)	2	1	15 0	7 5	45	7	9
Forest on hill (7)	1	—	5 5	5 5	60	1	1
Forest on Simbili (16)	1	1	8 0	8 0	—	—	—

\* Omitting early and late catches.

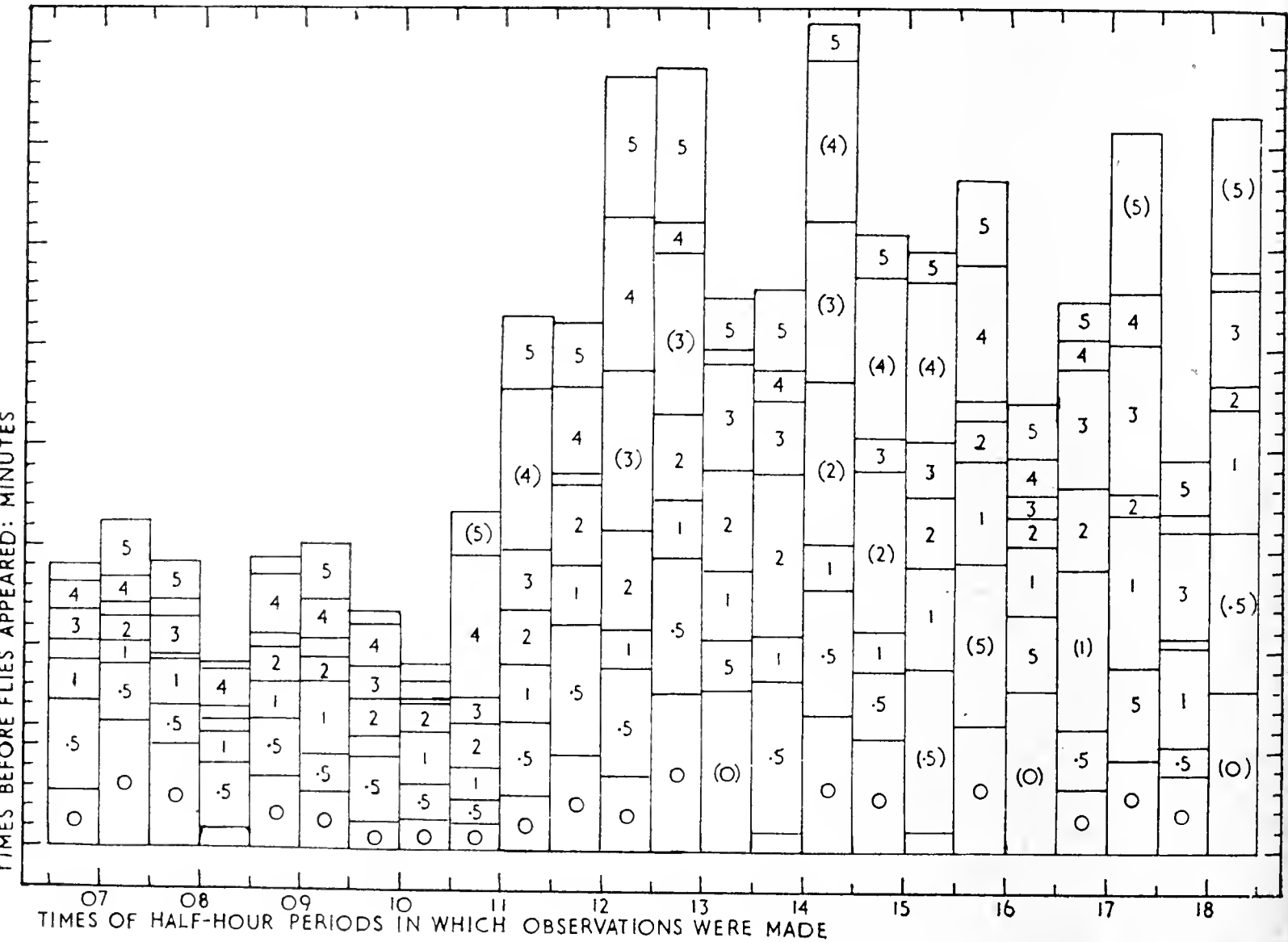


Fig. 2. Times that elapsed on various days from October 5 to 9 before flies appeared on the legs of collectors standing at six places between the river and Farangbaya. The figures in each column show distances from the river in hundreds of metres, and brackets indicate when no flies appeared.

GENERAL NATURE OF THE MOVEMENTS OF THE FLIES

Two searchers were placed at various points, with an observer who recorded the time which passed before a fly appeared. It was thought that this time might depend on the

number of flies present and give some indication of it. Table I and Fig. 2 show that the waiting time was very variable and, on the average, rather long in view of the prevalence of *S. damnosum*. In order to find out more about the insect's movements "standing catches" of 15 minutes and one hour were made and the time of capture of each fly was recorded. Standing catches have given some information about the behaviour of tsetse flies (Jackson, 1930; Buxton, 1955) with which *Simulium* are comparable to some extent, both being robust diurnal blood-sucking flies with a considerable range of flight. There is usually an initial high catch of tsetse, composed of flies which have followed the collector, flies which have approached him from a distance, and flies which have been waiting nearby. A prolonged sporadic catch follows, composed of flies which are moving about. The one-hour *Simulium* catches in open country (Figs. 3 and 4) showed no initial high figure but there was sometimes an initial pause, and it appears that the flies neither follow man nor are attracted to him from a distance. It is unlikely that the flies which bit at intervals during the one-hour catches had been waiting nearby, often for an hour or more, because there is very little shade in the grass-land. They were probably flying about at random and biting people near their path.

The average "time to appear" mentioned above, is not very different from the average interval between bites, and, in a long series of observations, would probably be half this interval plus a period caused by hesitation between appearance and biting. In the few catches made in forest away from the river (Fig. 3) more than half the flies were taken in the first 15 minutes. It is likely that these had been resting near the searchers and that flies do not move about much in this habitat.

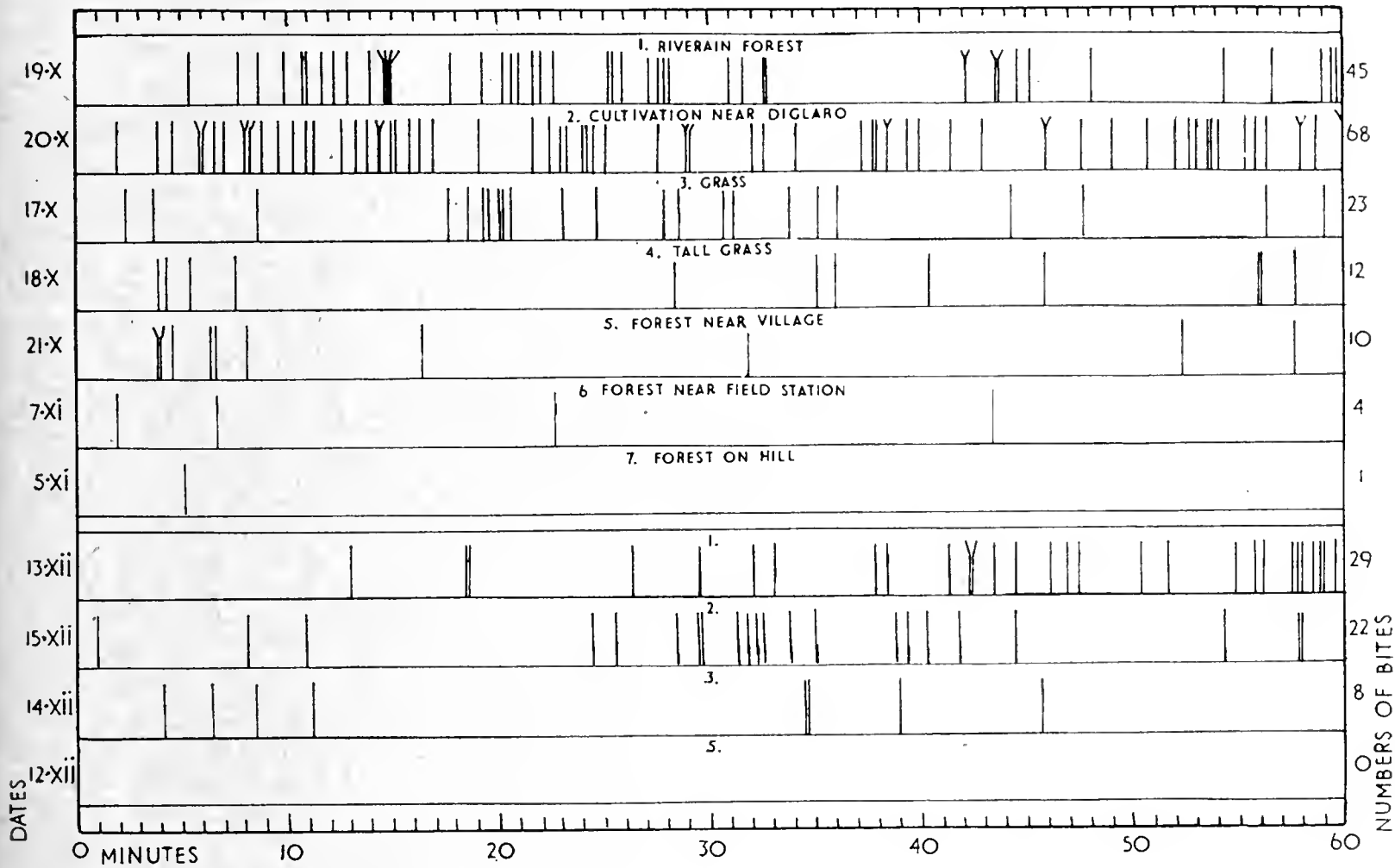


Fig. 3. Standing catches: Times of biting at several localities in the wet and dry seasons. Numbers of localities are those shown in Fig. 1.

TIME OF ACTIVITY

*S. damnosum* usually starts biting at dawn when nocturnal mosquitoes stop doing so. The time of biting has been found to depend partly on the physiological state of the flies (Lewis, 1956a, b), about twice as many nulliparous individuals occurring among flies caught in the afternoon as among an equal number caught in the morning. As a result many flies with a large fat-body were found in the afternoon, and there was a low infection rate in flies caught at this time (Table II). We do not know why nulliparous flies tend to bite late in the day but perhaps they are comparable to young mosquitoes which appeared to bite late in the night in Malaya (Colless, 1956, p. 102), or to *Glossina palpalis* R.D. with much fat which were found to be less hungry than those with little (Buxton, 1955).

TABLE II—The Results of Dissecting 869 Flies 84 of Which Contained Developing *Onchocerca*. Plain Figures show Percentages, Italics the Numbers of Flies Examined.

Localities	Morning			Afternoon		
	With much fat	With sausage-stage <i>Onchocerca</i> only	With vermiform <i>Onchocerca</i>	With much fat	With sausage-stage <i>Onchocerca</i> only	With vermiform <i>Onchocerca</i>
Near field station	31.7 82	6.3 96	12.5	69.9 83	5.0 80	6.3
Other areas	36.5 495	3.6 501	5.9	68.1 229	2.5 198	2.5
Total	35.8 577	4.1 597	6.9	68.5 312	3.2 278	3.6

In the rains the main biting period was either the morning or the afternoon, or was divided between the two with a lull at midday. The lull was thought to be associated with the frequent appearance of the sun at this time, but there are probably other reasons because the phenomenon is widespread. It was observed by the writer at Mvolo in the Sudan, and has been reported in various Simuliidae by Blacklock (1926), Buckley (1951), Crosskey (1955), Dalmat (1955), McMahon (1947), Rubtzov (1951) and other writers, and in the Tabanidae by Duke (1955) and others.

In the dry season, as Crosskey found in Nigeria, flies bit mainly in the afternoon. It is likely that few flies live long at this season and there is a preponderance of nulliparous ones which tend to bite after midday. Occasionally several flies bit after gleams of sunshine and puffs of wind but there was usually no obvious cause of bursts of activity. Flies were rather inactive on relatively cool winter mornings and very dull days.

#### THE EFFECT OF PEOPLE ON THE DISTRIBUTION OF FLIES

Flies were found to follow people for about five metres but not as far as 40 metres. They seldom appeared quickly when a walking person stopped, and no concentration of flies was noticed along paths. Any tendency to follow people seemed much too slight to affect the local distribution of the species, but Crosskey (1955) reported that in Nigeria flies tended to follow people accompanied by dogs.

Flies were reported, and noticed, to be often more numerous along quiet stretches of the Tonkolili than near rapids, possibly because many of the former were crossed by bridges which seemed to attract the flies. Some of these bridges were made of creepers suspended from overhanging trees and some were single tree-trunks felled across the river. People tended to linger at the bridges, particularly when they had broken down, and it is likely that some flies passing along the river waited in the vicinity until they succeeded in biting.

#### EXTENT OF MOVEMENT

Findings around Farangbaya were in accordance with the known considerable flying power of this species. Flies were found almost everywhere and did not tend to accumulate, or young ones to predominate, near breeding places. Catches in grass-land indicated extensive movement and were apparently unaffected by wind direction. Flies taken in some remote parts of the forest were found to include several nulliparous individuals with a large fat-body, which had probably come rapidly from distant breeding places. Some infected flies, found in wooded areas where no people or monkeys were seen, had evidently travelled far from the place of infection.

#### MALES

Two male flies were caught on a man's leg not far from the river, one near the Diglaro stream about 8 a.m. on 22nd October and the other near the Tonkolili on the morning of 22nd December.

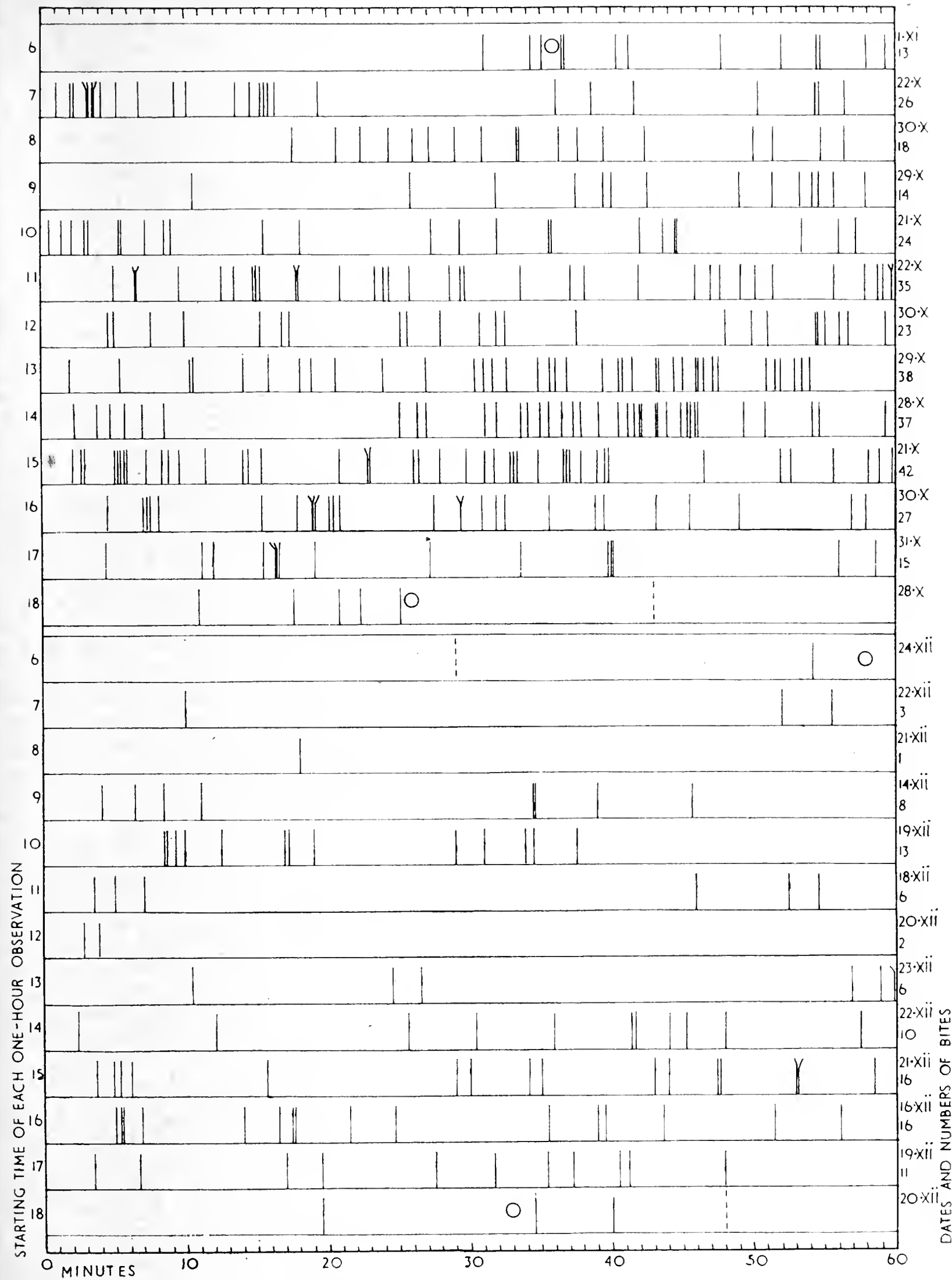


Fig. 4. Standing catches: Times of biting in the grass area near Farangbaya on various occasions at all hours of the day in the wet and dry seasons. Catches began between 8.30 and 9.40 a.m. Circles show times of sunrise and sunset and dotted lines the beginning and end of certain catches.

DISCOVERY OF THE PREY

It has been deduced that flies in grass-land are not attracted to man from considerable distances. Very few flies were caught in a patch of tall grass (Fig. 3) although there were many out of sight in open land a few metres away. Some of those caught seemed to have been blown in by puffs of wind and there was no evidence of olfactory attraction.

A striking feature of standing catches (Figs. 3 and 4) was the tendency for two or more flies sometimes to bite almost simultaneously after a considerable interval. This behaviour

was noticed by an independent observer who spoke of the flies attacking in waves. It is very unlikely that they move about in groups, and there was usually no obvious cause of these attacks, especially in riverain forest on a calm cloudless day. A similar phenomenon in mosquitoes has been discussed by Colless (1956, pp. 102, 109, 112) who suggests that direct attraction operates only over short distances and that mosquitoes resting near their prey may be activated in groups by slight local disturbances such as movements of people and puffs of wind. Possibly a local stimulus often activates *S. damnosum* and did so in the riverain forest catch of December 13th (Fig. 3). Towards the end of the hour the sun climbed to a position from which a few sunbeams reached the collectors and the neighbouring foliage, and the flies began to bite.

#### HESITATION BEFORE BITING

Apart from the hypothetical pause before short-range activation, there is a visible hesitation. Flies do not bite people until they stop walking and flies often hesitate a considerable time before biting a man who is standing still, even if he is unnaturally still in order to catch flies. They may probe the skin several times in different places or even probe shoes before they find the skin. While probing they are easily disturbed and may fly away before biting the same or another person. After a fly has appeared, several seconds or minutes may thus elapse before it bites. As a result, a stationary man who is bitten 60 times in an hour, for example, would receive fewer bites if he stood in 60 different places for one minute each.

On some days nulliparous flies bit more quickly than parous ones, and on the average more nulliparous than parous flies are able to suck blood from the collectors before being caught. Between October and December fresh blood was found in 33 per cent of 114 nulliparous and in only 18 per cent of 221 parous flies.

#### PREFERENCE FOR CERTAIN INDIVIDUALS

One collector who caught 284 flies was evidently more attractive to flies than the other who only caught 176. This preference was sometimes reversed when the men moved from tall to short grass or changed their relative positions.

#### CONTENTS OF THE OESOPHAGEAL DIVERTICULUM

In the northern Sudan the crop of *S. damnosum* often contains much solid material (Lewis, 1953) which may originate as dust on the fly's source of sugar. At Farangbaya the crop usually contained little or no solid material, possibly because the air in this wooded country during the wet season contains little dust. When a fly was offered sugar solution mixed with the local dust, powdered haematite, some of the latter quickly appeared on the bottom of the diverticulum where it could be seen through the body wall of the living fly.

#### FLIES AND VEGETATION

Although a few flies were to be found almost anywhere in the valley, *S. damnosum* was not numerous everywhere within a few kilometres of the river, as it is in open country in many parts of Africa. Flies were numerous in parts of the riverain forest strip and its immediate vicinity (Table I) and in certain cultivated and other areas with low shrubs; they were moderately numerous in grass-land west of the river; but they were few in the main forest area away from the river. Most catches in the main forest were made in small clearings, so the low apparent density was not simply due to bad visibility. The Sierra Leone Development Company's field station is in the main forest area; on a small grassy hill separated from the main grass area by about 200 metres of forest which seem to give it partial protection from *Simulium*. It appears that many flies spread from the riverain forest into the grass and scrub but that comparatively few pass on into the main forest where, as we have seen, they are evidently less active.

The standing catch in tall grass showed that flies penetrate it very slowly.

#### THE EARLY STAGES

Many streams flow into the Tonkolili but very few pupae of *S. damnosum* have been found in them. During the rains some pupae could usually be seen on trailing vegetation in fast water in certain parts of the Tonkolili, but not nearly as many as the numbers of adults would lead one to expect, and none could be found on accessible submerged vegetation. As the river fell a search was made for pupae on logs and other objects which had formerly



been submerged, but none were found except in a patch of *Eriocaulon* in a rapid, which appeared to be one of the main breeding areas. When pupae were kept in jars for the adults to emerge many did so in the early morning, some during the day, and very few at night.

### THE NUMBERS OF *S. DAMNOSUM*

The species occurred in moderate numbers (Table I) and was rather annoying at some places, but was far less troublesome than in many parts of Africa where hundreds can bite a man in an hour and several attack the same person simultaneously. Very little sunlight reaches the streams (Lewis, 1956b) which may therefore be too dark for the growth of algal food for the larvae. Flies diminished in December, in the early dry season, to about a third of their former numbers. The relative numbers in grass and forest remained about the same and the species was not confined to the river bank as it is in some countries at the height of the dry season.

### *S. DAMNOSUM* AND ONCHOCERCIASIS

On the average 9.7 per cent of the flies were infected with *Onchocerca*. The rate was rather higher near the field station (Table II), where many labourers gathered every morning, than in the area generally. The above-mentioned high proportion of parous, and also of infected, flies in morning catches does not necessarily indicate a greater risk at this time because often more flies bite in the afternoon. Out of 84 infected flies examined 40 contained sausage-stage larvae (there were eight mixed infections) and 52 contained vermiform larvae. Of those with vermiform larvae 30 were infected in the head and five in the abdomen. The average number of vermiform larvae was 4.5 per fly and 3.1 per infected head. In one unusual instance 10 vermiform larvae were found in the abdomen. *Onchocerca* were often seen to emerge from the head of a fly at the beginning of a dissection. They sometimes appeared to emerge in a pair, one on each side of the proboscis, occasionally followed by a second and third pair.

It is appropriate to consider whether our present knowledge of the fly can help to explain why there is so little blindness at Farangbaya. It is difficult, however, to estimate how many times a day the various members of the community are bitten by flies because this does not depend simply on the number of flies present. People who spend much time indoors or in the forest away from the river tend to be bitten much less than people who frequent bridges, exposed washing places and river-side farms. People who keep moving when out of doors, or remain stationary for a considerable aggregate period composed of short stops, may escape attack when those who are stationary for a considerable time receive many bites. The higher infection rate in flies biting in the morning tends to increase risk of infection at that time, and there is some evidence, as noted above, that on occasions young flies bite more readily than old ones so that risk is somewhat reduced. Apart from these uncertainties as to the number of bites and infective bites received, however, it is likely that the limited number of flies and the extensive non-riverain forest limit the transmission of onchocerciasis to some extent.

Control of *S. damnosum* should be easy but it will be necessary to decide how much to do. It may be that very incomplete control would eliminate what little blindness there is. Kershaw and others (1954) have suggested that repeated temporary reduction of a vector might result in an absence of eye changes, and Gordon (1955) thought that even a small reduction in infective density of a vector might conceivably eliminate blindness.

It is neither necessary to exterminate *S. damnosum* in the valley nor is it possible to do so effectively because it would certainly return from other valleys. Treatment of the Tonkolili, but not its tributaries, with DDT at the end of the dry season and at fortnightly intervals during the rains would probably be more than enough to eliminate onchocercal blindness and to minimize annoyance by the flies, and in due course it may prove that only occasional treatments are necessary. It would probably be wise to preserve large areas of thick forest as far as possible. Dimethyl phthalate was useful as a repellent but had to be applied much more often than in a drier area like the southern Sudan, because it was often removed by excessive sweating and long wet grass. D.M.P. could usefully be carried by foremen in charge of gangs of labourers. The protective value of long trousers was shown by a resident near Simbili who habitually wore them and had not noticed the existence of *Simulium*.

## ACKNOWLEDGMENT

I am indebted to Professor V. B. Wigglesworth, C.B.E., F.R.S., for drawing my attention to the work of Voïnov and Epure.

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## DISCUSSION

B. HOCKING. Have you any views on the significance of the difference between the anterior and posterior tergites of the abdomen?

D. J. LEWIS. Rubtzov has suggested that the weak chitinization of some anterior abdominal segments of *Simulium* is an adaptation which allows for distention during a blood meal, and I agree with him.

# The Biology and Control of Black Flies (Diptera: Simuliidae) in Canada<sup>1</sup>

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## ABSTRACT

*Development of the Canadian north led to increased interest in black flies. Fifty-eight species have been recorded and described for Canada. Simulium venustum Say and Prosimulium hirtipes (Fries) are the major pests of man. Simulium arcticum Malloch is a serious livestock pest on the prairies. In Canada, the species attacking man are not vectors of any known disease, although those attacking birds transmit protozoan and nematode parasites. Progress to 1956 of biological studies is summarized, including distribution; seasonal succession; geographical, meteorological, and ecological factors affecting the development, habit preferences, and level of infestation of egg, larva, pupa, and adult; assessment of larval and adult populations; laboratory rearing; diurnal activity; feeding, mating, ovarian development, and oviposition, with particular reference to biting habits and blood meals; migration of larvae and adults; natural longevity; food, predators, and parasites; transmission of animal parasites; and, host reaction and immunity to bites.*

*An efficient method of larval control was developed in northern Manitoba in 1947. DDT in fuel oil solution is applied at one part of DDT to ten million parts of water for 15 minutes at the point of application. Its application in prairie and forested regions is described. Aerial sprays to control adults are also discussed.*

## INTRODUCTION

Black flies are serious pests of man and animals in most regions of Canada. The heaviest infestations occur in the forests of northern and Eastern Canada, as well as in the Rocky Mountains. In pulpwood cutting areas of Eastern Canada, the adverse effects of these insects on the efficiency, morale, and recruitment of labour, as well as on the implementation of a plan of year-round operations, has exemplified the necessity for their control. In subarctic terrain in Labrador during the biting-fly season, a decreased efficiency of a small mobile military force was caused by irritation, painful reactions, and infections from bites; loss of sleep; general discomfort; entrance of flies into ears and nose; and, psychological responses.

In 1947, extensive research on biting flies was organized as a joint project of the Entomology Division, Canada Department of Agriculture, and the Defense Research Board. A survey of northern insects was assigned to the Systematic Entomology Unit, and the biological and control studies to the Veterinary and Medical Entomology Unit of the Entomology Division. The Pulp and Paper Research Institute of Canada entered the program in 1952. The co-operation between federal Government departments, the Pulp and Paper Research Institute, and universities, has resulted in a great increase in knowledge of the biology of biting flies, and in the development of effective control measures.

In this paper, a brief picture of the present position of research on black flies in Canada is presented. The early studies of Hearle (1929, 1932, 1934, 1938), Cameron (1918, 1922), and Twinn (1933, 1936, 1939) on Canadian black flies are well known. Twinn (1950, 1952, 1956) has reviewed the studies conducted by several workers since World War II. Field studies in the forest lands in Eastern Canada, and on the prairies in Western Canada, are recorded in the Progress Reports of the Veterinary and Medical Entomology Unit, and are being prepared for publication.

Studies of the distribution of black flies in Canada are to be presented at this Congress by Shewell and Fredeen. The cytological studies of Rothfels and Dunbar (1953) deserve mention, particularly for their value in clarifying difficult taxonomic and phylogenetic problems.

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## BIOLOGY

## SEASONAL SUCCESSION AND ABUNDANCE

A striking feature of field studies in any particular area at the beginning of the black-fly season, is the clearly defined nature of the larval, pupal, and adult populations. As the season advances, considerable overlap of generations occurs as a result of late emergences of the first generation from small, spring-fed streams; the entrance of black flies from areas nearby; the occurrence of more than one ovarian cycle in some species; and, the availability of food. The small permanent river, interrupted occasionally by falls, with many rapids and rills, a graded stony bed, and abundant emergent and border vegetation, is the adolescent stream by Dalmat's (1955) classification. It is the type watercourse for studies of the seasonal succession of black-fly populations.

There are two peaks of abundance of black flies in the first generation. These represent species overwintering as larvae and those overwintering as eggs. *Prosimulium hirtipes* (Fries) is the characteristic species of the first peak and *Simulium venustum* Say of the second. These are the major species biting man. In adolescent streams, the population that arises from overwintering eggs is by far the largest, best defined, most dramatic in appearance, and most important in relation to the timing of control procedures. Levels of infestation in rills of these water-courses may reach 5 million larvae to the square metre of river bed, and the contribution that even a short section of such a river makes to the adult population is very significant. In smaller, permanent streams, with stony beds and banks, many rapids and waterfalls, i.e. the young stream of Dalmat, the population of black flies arising from overwintering larvae is the largest, and *P. hirtipes* is the dominant species. *S. venustum* replaces *P. hirtipes* in the early summer. These streams join into the adolescent stream and are more numerous at the upper reaches of rivers and consequently at higher altitudes.

The seasonal succession of the commoner species of black flies in Canada is shown in Fig. 1. On the north shore of the St. Lawrence River, *Cnephia invenusta* Walker is the first black fly to emerge as soon as the rivers are ice-free, and it has a short fly season. However, Ide *et al.* (in preparation) report that *Simulium furculatum* Shewell and *Cnephia mutata* (Malloch) are the first species to emerge in northern Manitoba.<sup>4</sup>

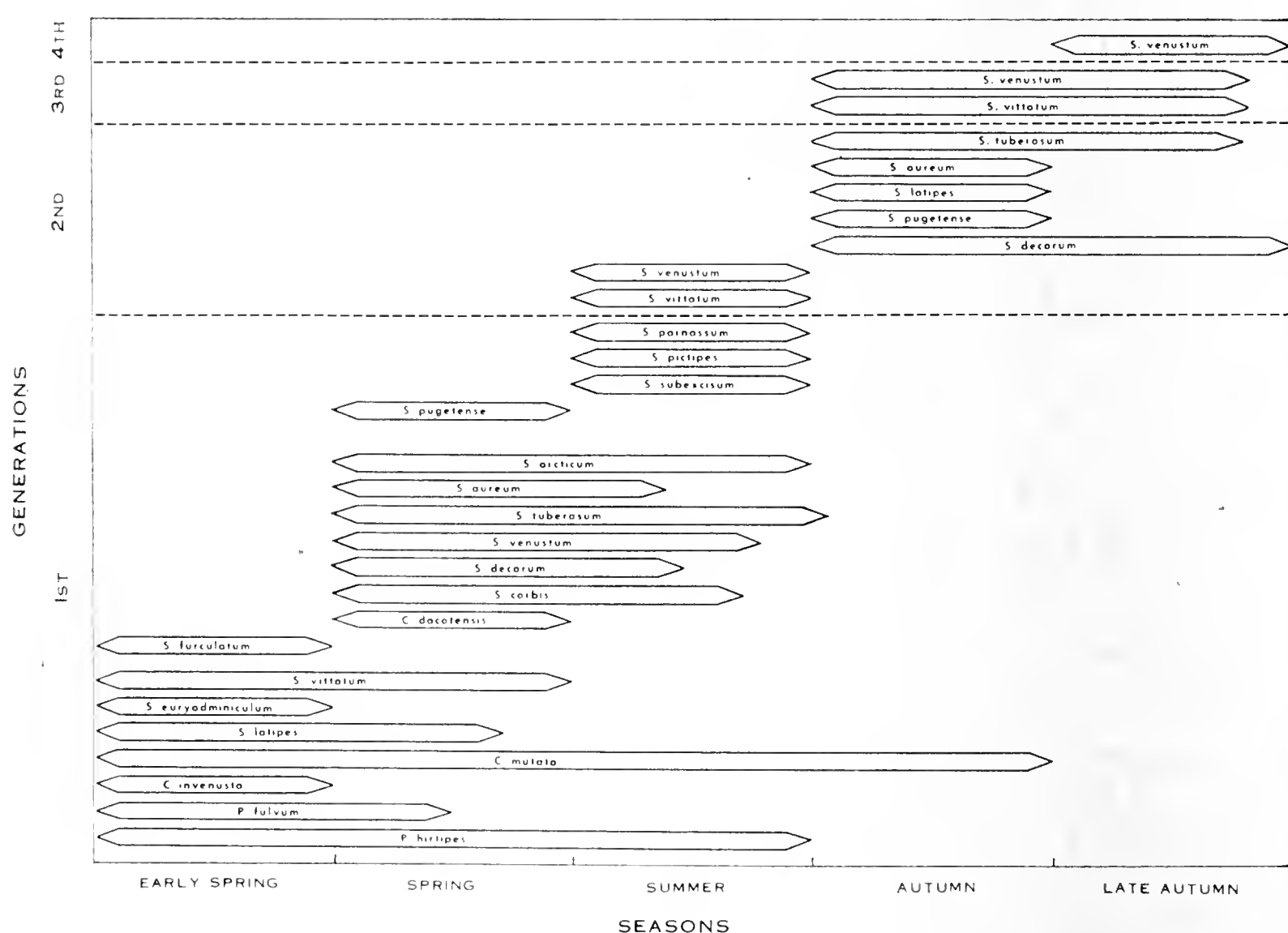


Fig. 1. Seasonal succession of black flies in Canada.

<sup>4</sup>Twinn *et al.* (1948) recorded that the first species on the wing at Churchill, Man., in 1947 was *S. vittatum* Zett. (Ed.).



Certain species, such as *Simulium pictipes* Hagen and *Simulium parnassum* Malloch have a long period of larval development, and appear concurrently with second and third generations of other species. *S. venustum* requires 15 to 18 days for larval development, and 5 to 8 days for pupal development, in waters at temperatures of 55 to 60°F. The total period from egg to egg is approximately 30 days, which permits three or four generations to develop in a year in southern Canada. The entire black-fly population rapidly declines in the late summer, as many watercourses dry up, the levels of rivers fall, the character of the water currents change, and stones in the streams become covered with algae.

#### EMERGENCE

Considerable information has now accumulated on the dates of emergence of black flies in many parts of Canada. Ide *et al.* (*op. cit.*) have constructed maps of isophanes, for the common species, from comparable watercourses in continental regions. Their data, corrected for altitude, season, and coastal proximity, shows, for early species, a 6½-day retardation factor in the commencement of emergence per degree of latitude northward along the meridian between Algonquin Park, Ont., and Churchill, Man. There is a 4½-day retardation factor per five degrees of longitude eastward along the parallel between Whitehorse, Y.T., and Goose Bay, Lab. The seasonal succession is similar, even though the dates of emergence change in different parts of the country. In northern Canada, however, there may be only one or, at the most, two generations in a year.

#### WATER TEMPERATURE AND STREAM TYPES

The topographic features of the watershed are of first importance in determining the minimum water temperature, rate of warm up, rate of development, time of emergence, and species present. The heaviest infestations of *Simulium* larvae are found in shallow rills of permanent streams, particularly at the outlets of small lakes, ponds, calm reaches, and reservoirs. In these places, the water warms rapidly in the spring. Hocking and Pickering (1954) suggested that the favourable nature of the efflux of streams from lakes was related to abundance of food micro-organisms, and to the ease with which gravid females could locate such regions for oviposition.

In spring-fed, forest streams in which the temperature may never exceed 50°F., larval development requires almost the entire summer, and small numbers of spring species, such as *P. hirtipes* and *C. mutata*, may continue to emerge until the fall. Rivers from large lakes warm up more slowly than those from smaller lakes. This is related to the time of break up and disappearance of ice, which in turn is affected by wind velocity and direction. Once average minimum temperatures exceed 40°F., black-fly development progresses rapidly. However, *P. hirtipes* will grow and pupate at temperatures as low as 35°F. Optimum temperatures for development are 55 to 65°F., whereas mean water temperatures over 70°F. are generally unfavourable.

The black flies associated with a particular type of watercourse are shown in Table I. Adolescent streams provide the most suitable conditions for larval development. The genera *Gymnops* and *Prosimulium*, however, prefer forest streams in upland regions.

#### pH AND OXYGEN CONCENTRATION OF STREAMS

Black-fly streams are usually slightly alkaline with a pH of 7.0 to 7.5, but development can occur in waters having pHs from 5.8 to 8.5. Current velocity rather than oxygen concentration is the important factor determining distribution of larvae. Heavy algal growth may cause diurnal fluctuations in oxygen tension of the waters without affecting the larval distribution.

#### LARVAL ATTACHMENT

A current velocity ranging from 1/3 to 1 ft. per sec. is preferred by the larvae of most species: *Prosimulium* species as well as *S. pictipes* and *Simulium decorum* Walker do occur in waters where the current velocity is greater than 1 ft. per sec. Hocking and Pickering (*op. cit.*) regard a steep velocity gradient as providing the mechanical force for moving larvae from the main flow towards sites favourable to attachment. Larvae cannot swim and are helpless when freely suspended. Tactile and visual stimuli, however, operate to complete the attachment process. The energy for most of the locomotory activity of the larva is provided by the current which also guarantees abundant food and oxygen.



TABLE I—Association of Black-fly Species with Stream Types in Canada.

Stream type	Infant	Spring-fed	Young	Adolescent	Mature and old rivers	Dams and sluices
Level of infestation	Low	Very low	Moderate	Very high	Moderate	High
Black-fly species	<i>P. hirtipes</i>	<i>P. hirtipes</i> <i>P. fulvum</i> <i>G. holopictus</i>	<i>P. hirtipes</i> <i>P. fulvum</i> <i>G. holopictus</i>	<i>P. hirtipes</i> <i>P. decemarticulatum</i>	<i>P. multidentatum</i>	
	<i>C. mutata</i>	<i>C. mutata</i>	<i>C. mutata</i> <i>C. emergens</i> <i>C. dacotensis</i>	<i>C. mutata</i> <i>C. invenusta</i> <i>C. eremites</i>		
		<i>S. venustum</i> <i>S. latipes</i>	<i>S. venustum</i> <i>S. tuberosum</i>	<i>S. venustum</i> <i>S. latipes</i> <i>S. euryadimaniculum</i> <i>S. vittatum</i> <i>S. tuberosum</i> <i>S. corbis</i> <i>S. decorum</i> <i>S. arcticum</i> <i>S. aureum</i>	<i>S. venustum</i> <i>S. pictipes</i> <i>S. rugglesi</i> <i>S. tuberosum</i> <i>S. aureum</i> <i>S. decorum</i> <i>S. arcticum</i>	<i>S. decorum</i>
			<i>T. tibblesi</i>			

Hocking and Pickering showed that larvae attach most commonly to surfaces over which the stream flow is laminar. This is generally the situation on the upstream surfaces of logs, stones, or upright blades of grass. The authors have found the greatest number of larvae attached to the upstream face of plates inclined 20 to 50° to the current. Hocking and Pickering calculated that the number of larvae attaching per unit of length of blades of grass was approximately proportional to the sine of the angle of inclination to the current.

Pupae prefer to attach in more protected locations provided there is constant turbulence. This situation exists on the downstream surfaces of logs, stones, and blades of grass. Turbulent micro-currents would provide a greater access to oxygen for the pupal respiratory filaments.

LARVAL MIGRATION

After hatching from the egg, the first-instar larva floats in the water, and is carried passively by the current to swifter waters. A silk-like adhesive thread is secreted, and serves as the initial attachment to the stream substratum. Firm attachment is maintained by the anal hooks. After the initial attachment, the larva migrates to a position where the character of the current is optimal. Changing stream characteristics or other disturbances cause the larvae to release the clasp of the anal hooks and be carried by the current, held only by the thread of silk along which they can migrate back to their original position. Often they release themselves from the thread and reattach at a new site. Rubzov (1939) demonstrated a diurnal migration of larvae. The authors frequently observed that assessment cones, which are discussed later, became covered with larvae more rapidly at night. In the daytime, the larvae congregated in masses and at night migrated downstream.

ASSESSMENT OF LARVAL POPULATIONS

Ide (1940) and later Davies (1950) placed cubic yard emergence cages over the stream bottom to sample populations of emerging flies, a method also used by the Entomology Division in northern Canada (Twinn, 1950). These cages were examined daily or even hourly. While this method gives precise information on the emergence and succession of species, it is not practical for assessment during control operations. Arnason *et al.* (1949), sampled known areas along transects to estimate larval infestations during treatments of the Saskatchewan River. A 5-minute stone count was used by Brown (1955) for pre- and post-treatment assessments of aerial applications of larvicides near Baie Comeau, Quebec. In 1955, a new method was developed by the junior author. Hollow metal cones, with a base diameter of 10 cm., a height of 20 cm., and an apex angle of 30°, were painted white and attached by wire to logs, stones, or low vegetation, and suspended in the streams. These cones provided excellent attachment surfaces and were preferred to stones or trailing

grasses. First-instar larvae are strongly photopositive and prefer light-coloured surfaces. The cones were of value for the following purposes: determining the level of infestation at a particular locality; estimating the growth rate of larvae; determining periods of migration of small larvae throughout watercourses from oviposition areas; determining periods of peak abundance of larvae and pupae; indicating streams suitable for larval growth; and, assessing larval control methods.

The assessment of migration of larvae as an indication of larval populations is new, and the cone technique was particularly suitable for the conditions under which studies in pulpwood cutting areas were conducted. Fig. 2 illustrates the seasonal changes in numbers of black-fly larvae at a sampling station, as determined by cone counts. Two cones were placed in the stream. The number of larvae attached to these were counted at intervals. One of the cones was cleaned following each count, while larvae were permitted to accumulate on the second.

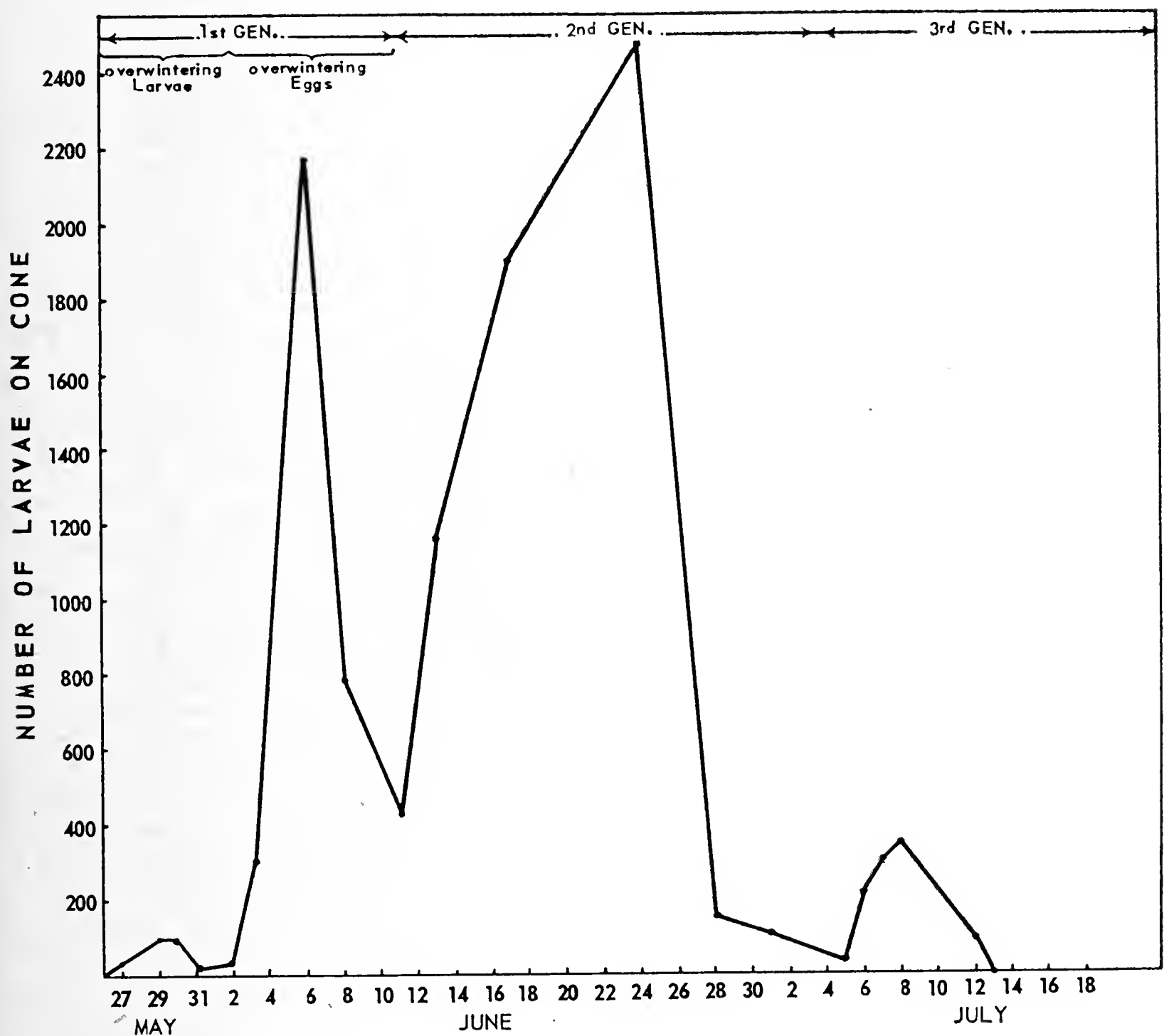


Fig. 2. Levels of infestations of black-fly larvae at a sampling station in Trembley Creek, near Baie Comeau, Que., May 26–July 18, 1955.

#### ASSESSMENT OF ADULT POPULATIONS

The studies of Davies (1951), and Hocking and Richards (1952), have led to the development of a standard, landing-rate count for assessing adult abundance. A 12-inch square of dark blue serge cloth, divided by white lines into 9 squares, is placed on the lap of an observer and, after a 2-minute wait, the number of flies landing during the next minute is counted. When possible, three simultaneous and three successive counts at 2-minute intervals are made. The use of dark blue cloth was suggested by the studies of Davies (*op. cit.*), and Brown (1951) on factors in the attraction of biting flies to man.

## REARING

Fredeen (personal communication) has recently developed a rearing technique for several species of black flies. Previously, numerous techniques had been tried with only partial success. The development of Fredeen's rearing technique should lead to important advances in the study of black-fly biology and physiology.

Davies (1953) reported success in keeping adults of *S. venustum* alive in special glass tubes for up to 63 days. However, only 4 per cent of the initial numbers lived this long. The authors were unsuccessful in similar attempts. Many modifications were tried but failed. Flies collected in the field or reared from pupae did not survive more than 48 hours. Progress in studies of the susceptibility of black flies to insecticides, and of the transmission of parasites, is dependent on the development of a technique for establishing self-perpetuating colonies of black flies.

## DIURNAL ACTIVITY

Factors that affect the diurnal activity of adult black flies have been studied by Davies (1952). To extend the study on black-fly behaviour, and to obtain information on the best time to apply adulticides, the authors made a series of 24- and 48-hour checks simultaneously in different localities and in consecutive summer months. A complete, portable meteorological station was set up and hourly records made of climatic conditions, light intensity, landing, biting, and sweep counts.

The black-fly activity on June 23, 1955, at Brisson Creek, Baie Comeau, Que., is shown in Fig. 3. Fig. 4 shows the activity during a 48-hour period at a forest station. When the light intensity fell below 1 foot candle, black flies disappeared dramatically to resting places in trees and shrubs. The flies moved at night towards the tops of trees and came down in the daytime. During the day, they rested on the underside of leaves of trees and border vegetation. Relative humidity, wind velocity, temperature, and changing light intensity are the four variables affecting activity. These, as Davies showed, may act together or antagonistically. In the morning and evening, these variables operate in such a way as to cause the maximum stimulus to biting-fly activity. Provided temperatures were not below 50°F., wind velocity not greater than 2 m.p.h., and the relative humidity not below 70 per cent, changing light intensity in the morning and evening, or from direct sunlight to skylight, was the initial and important factor determining activity. Overcast periods favoured increased activity and zones between the shade of vegetation and direct sunlight were preferred. The percentage of black flies biting was higher in the morning and early evening, and decreased during rainy and windy periods, and when the relative humidity was over 95 per cent. Of interest here are the recent studies of Harker (1956) on factors controlling diurnal rhythms in the cockroach. He found that alternating lightness and darkness impresses the rhythm but temperature can modify it. The physiological mechanism of this process is thought to operate through the ocelli, to neurosecretory cells in the suboesophageal ganglia. The observations and conclusions, on the activity of black flies, apply only to woodland species. Fredeen (personal communication) has some evidence that other environmental factors, e.g. changing barometric pressure, may be important in determining the activity of prairie species, e.g. *Simulium arcticum* Malloch will attack cattle when wind velocities greatly exceed 2 m.p.h.

## FEEDING, MATING, OVARIAN DEVELOPMENT, AND BLOOD MEALS

Both male and female black flies feed on nectar as a source of energy needed for flight, and the females require protein to complete egg development. The observations of Cameron (1922) on *S. arcticum*, and Jobbins-Pomeroy (1916) and Wu (1931) on *S. venustum*, showed that the immature ovaries in adults reared from pupae, underwent rapid maturation after a blood meal. A blood meal was, therefore, generally regarded as essential for ovarian maturation. Recent studies have cast doubt on the generalization that blood is the single source of the required protein. The authors found that the stage of egg development at emergence not only varies between different species, but within one species. Newly emerged females of *S. decorum* and *Simulium vittatum* Zett. with mature ovaries were repeatedly observed ovipositing within 48 hours of emergence. Females of *P. hirtipes* with partially developed eggs on emergence were observed ovipositing before the fly became a biting pest. Evidence is accumulating which suggests that the first ovarian cycle is completed in some species with protein retained from the larval and pupal stages,

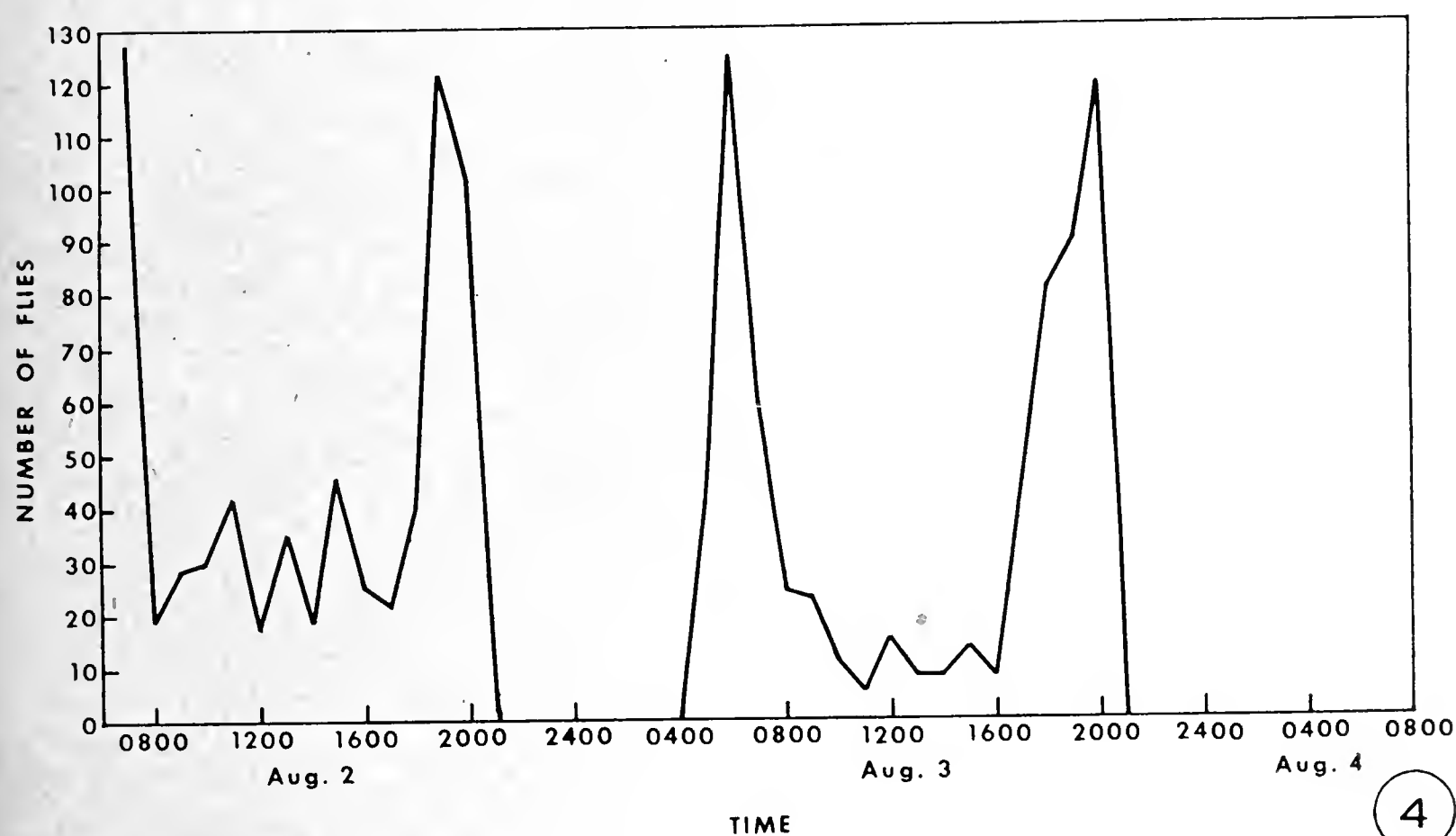
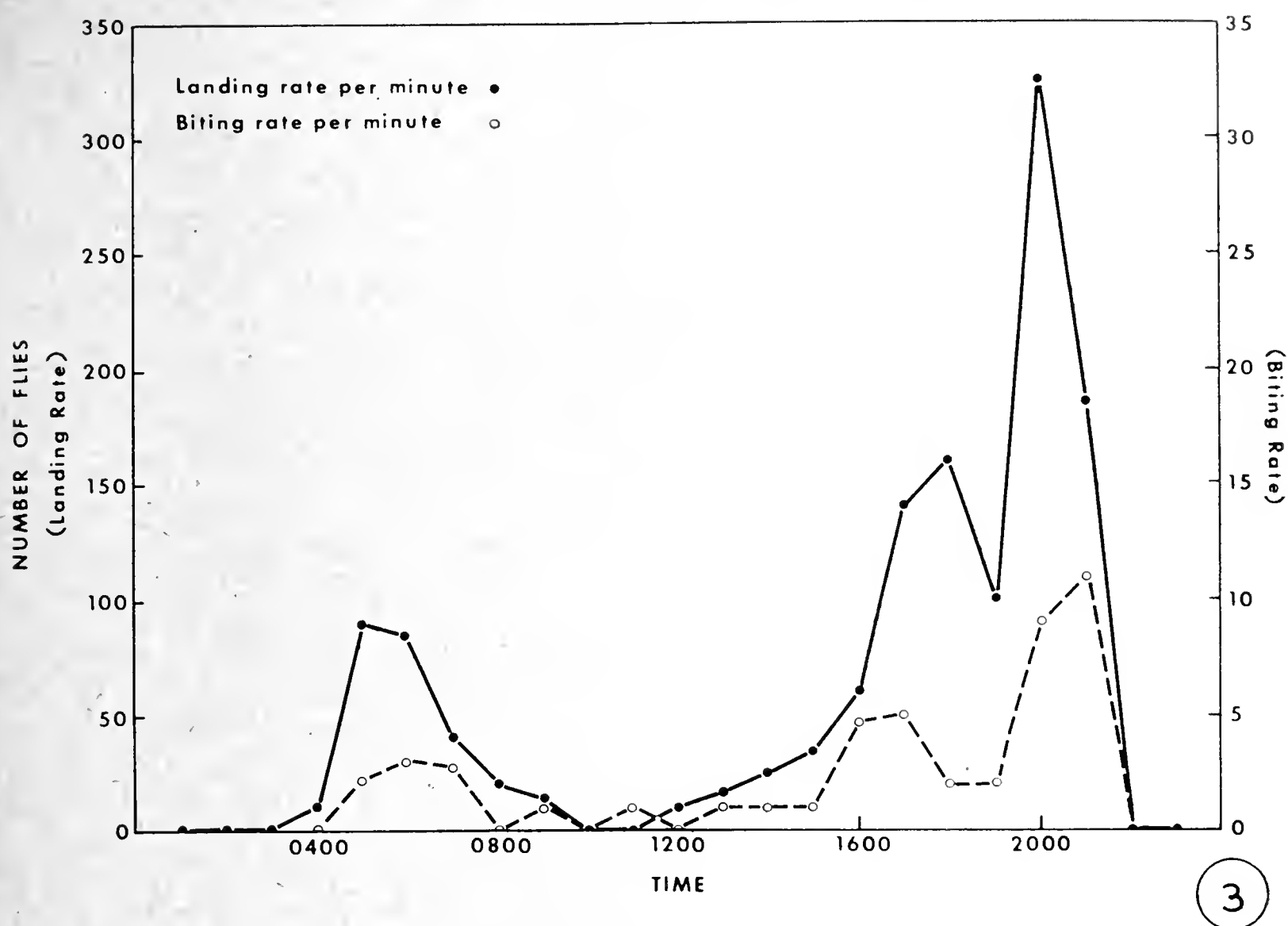


Fig. 3. Activity of black flies at Brisson Creek, near Baie Comeau, Que., during a 24-hour survey on June 23/24, 1956, showing mean landing rates on 9 in. x 9 in. blue cloths and mean biting rates on bare forearms recorded by three observers.

Fig. 4. Activity of black flies at a station in the spruce forest near Baie Comeau, Que., during a 48-hour survey, August 2-4, 1956, showing total number of flies collected by three observers in sweeps at hourly intervals.

and that if a blood meal is obtained after oviposition, a second ovarian cycle may be induced. Simuliidae have polytrophic ovarioles, and multiple gonotrophic cycles have been recorded for *Simulium damnosum* Theobald by Wanson and co-workers (1946, 1950) and Lebied

(1950). An interesting corollary is that factors affecting the storage of protein nutrient in the larval fat body may modify adult biting habits. Species such as *Cnephia dacotensis* (Dyar and Shannon), *C. emergens* Stone, *C. eremites* Shewell, and most *Gymnopaia* species, possess thin, poorly-dentate mandibles and hirsute galeae and do not bite. The females are gravid on emergence.

Black flies are known to feed on a variety of animals but information on biting preferences is incomplete. Serological methods were developed and used by Eligh (1952), West and Eligh (1952), Hall *et al.* (1953), Downe and West (1954), and Downe and Morrison (1957), to determine the source of blood. The sources for some of the common species are shown in Table II. Shewell (1955) has pointed out the important morphological difference between species that feed on mammals and those that feed on birds. Species with simple claws prefer mammals whereas those with chelate claws feed on birds. The special nature of the claw appears to be an adaptation for walking over the barbs of feathers.

TABLE II—Sources of Blood-meals for Some Common Species of Black Flies in Canada.

Man	Mammals (cattle, sheep, deer, etc.)	Birds (domestic and wild ducks, etc.)
<i>P. hirtipes</i>	<i>P. hirtipes</i>	<i>S. rugglesi</i>
<i>P. fulvum</i>	<i>P. fulvum</i>	<i>S. venustum</i>
<i>S. venustum</i>	<i>C. mutata</i>	<i>S. aureum</i>
<i>S. parnassum</i>	<i>S. venustum</i>	<i>S. croxtoni</i>
<i>S. arcticum</i>	<i>S. arcticum</i>	<i>S. latipes</i>
<i>S. vittatum</i>	<i>S. vittatum</i>	<i>S. jenningsi</i>
<i>S. corbis</i>	<i>S. luggeri</i>	<i>S. euryadminiculum</i>

In mating behaviour, black flies can be divided into two major groups. *C. dacotensis* and perhaps *C. eremites* belong in the first group. They have no mating flight or swarm and copulate soon after emergence. Downes (1955) suggested that this habit may be related to the fact that the eyes are not clearly divided into large upper and small lower ommatidia as is usual in other species. In the second group, of which *P. hirtipes* and *S. venustum* are examples, an organized mating swarm occurs. There is some evidence which suggests that certain species may be parthenogenetic, as was reported by Davies (1950) for *C. mutata* in southern Ontario. Cytological work (Rothfels, personal communication) substantiated this observation.

#### OVIPOSITION AND DIAPAUSE

Oviposition usually occurs between the late afternoon and early evening following bright sunny days. On overcast days or with sudden weather changes, it may be earlier. Fredeen *et al.* (1951), Giglioli (1955), and Davies (in prep.), have each recorded details of time and locale for many species. Calm, steadily-flowing waters at lake outlets, which are above fast flowing waters, are the preferred sites, and selection seems to be by visual means. Early in the season, eggs are deposited on stones and later on floating vegetation. We observed two main methods of oviposition. In one, eggs are released singly or in small batches, while the female is in flight close to the water surface. The eggs of most species in this group, e.g. *P. hirtipes*, have a summer diapause and hatch in the autumn when stream temperatures fall. *S. arcticum* is an exception for it has an overwintering egg stage. In the other group, e.g. *S. venustum*, *S. decorum*, *S. pictipes*, *S. vittatum*, eggs may be deposited singly, in batches, in irregular strings, or as a compact mass, by the female while it is resting on a suitable surface. *S. decorum* has been observed ovipositing while in flight. The eggs of the species in the second group generally have a winter diapause and hatch in the spring when stream temperatures rise. However, some of the *S. vittatum* population may overwinter as larvae, while *C. dacotensis* has both a summer and winter diapause.

#### FLIGHT RANGE AND DISPERSION

Wind is an important agent in dispersing adult black flies as reported by Cameron (1922) for *S. arcticum*. On the prairies, strong winds carried black flies distances of up to 140 miles. The authors have observed that *S. venustum* and *P. hirtipes* may be carried up



to 5 miles from the nearest breeding places in forested areas. Certain species can be carried considerably further in the "black-fly cloud" and in vehicles, but these methods are of minor importance. Fredeen *et al.* (1953) used mass tagging with  $P^{32}$  in the late larval stages in studying flight range. They achieved little success in field recoveries. However, this work indicated the feasibility of radioactive tagging and its usefulness in flight range studies, when better collecting methods are developed. The extensive use of light traps and animal traps has not been attempted.

#### TRANSMISSION OF PARASITES

The transmission of animal parasites by black flies has been studied in Canada by Fallis, Davies, and Vickers (1951), Anderson (1954), and Anderson and Fallis (1955). They have added considerably to knowledge of the transmission of blood protozoa and filaria in domestic and wild ducks. *Leucocytozoon simondi* is transmitted by *Simulium rugglesi* Nicholson and Mickel and *Eusimulium* species. *Ornithofilaria fallisensis* is transmitted by a number of ornithophilic species as well as by *S. venustum* and *S. parnassum*. So far, in Canada, black flies have not been implicated in the transmission of parasites or viruses to man. However, further investigation is needed, particularly as man establishes himself in the heavily infested areas of northern Canada.

#### HOST REACTION AND IMMUNITY TO BITES

The psychological reactions to the presence of black flies are as important as the physiological effects of their bites. Hocking (1952) described a sequence of reactions in man. A removal reaction is initiated by the continual noise and impact of flies on the face and body as well as by the bite. Continued exposure leads to this reaction being evoked by insects alighting on the skin, and, in some individuals, an hallucination develops to nonexistent insects. The final stage is the running away reaction. This is frequently observed in animals. Reduction in efficiency and morale occurs earlier from psychological than from physical causes.

The reaction to black-fly bites is more serious for most individuals than that to mosquito bites. Although the penetration of the skin is usually painless, the mechanical trauma and loss of blood are greater than from mosquito bites. Children severely bitten around the face and neck often present an alarming sight because of profuse bleeding from the skin lesions, but do not complain immediately of pain or itching.

An immediate reaction with wheal formation does not occur in the unsensitized person. A 5- to 10-day period of first exposure to black-fly bites is required for sensitization. In a sensitized person, 24 to 48 hours after being bitten the delayed reaction causes the formation of an itchy, weeping papule that irritates for at least a week and sometimes up to a month. Scratching or rubbing of this lesion causes a wheal to form and sometimes secondary infection. The delayed reaction is probably due to the injection of slow-acting toxins rather than the release of histamine by the host. Hutcheon and Chivers-Wilson (1953) obtained small quantities of histamine and an anticoagulant from black flies, but they point out that the generalized toxic reactions to bites must result from other substances in the salivary secretion.

If a hyper-sensitivity develops, black-fly bites may lead to a generalized allergic dermatitis. However, it is more usual, after prolonged exposure to bites, to develop a degree of immunity. The development of a painful lymphadenopathy, particularly of the posterior auricular and occipital lymphatic chains, is a characteristic sequel of black-fly bites and leads to the well known "black-fly stiff neck." Fortunately, this reaction is one of the first features against which immunity develops. As the season advances, the reaction to bites becomes less severe, but the immunity acquired in one season does not necessarily provide protection during the next. The whole question of sensitization and immunity to black-fly bites needs further investigation. Dr. West, Queen's University, has already initiated studies in this field. The determination of blood levels of histamine and changes in the blood eosinophil and leucocyte counts during exposure to black flies would be valuable. Stokes (1914) is believed to be the only investigator who has published details of the clinical and pathological aspects of black-fly bites.

#### OUTBREAKS

Cameron (1922), Millar and Rempel (1944), and Rempel and Arnason (1947) have given detailed accounts of periodic outbreaks of *S. arcticum* in Saskatchewan that caused

serious losses of livestock. The sporadic nature of these outbreaks, Remple and Arnason point out, is dependent on a complex of biological and ecological factors not yet understood. Black flies carried long distances across the open prairies by the wind may not return to their breeding areas. Re-infestation of the streams must be made by flies remaining close to the rivers and several years may be required for high levels of infestation to again build up.

The most common symptom observed in cattle attacked by *S. arcticum* is lymphatic swelling in the throat and dewlap. Breathing becomes heavy and stertorous, associated with muscular tremors. Death occurs in from 15 minutes to 2 hours after the first observable symptoms. Recovery, if it occurs, is complete in 24 to 48 hours. Post-mortems revealed numerous ecchymotic spots at the site of the bites, as well as anasarca and multiple petechial haemorrhages on all viscera and serous membranes. Lymph glands were particularly enlarged and oedematous. Millar and Rempel (1944) concluded that shock was the cause of death, resulting from the direct toxic action of the foreign protein injected by the insects, without previous sensitization. However, the pathogenesis of this condition in cattle is still little understood.

## CONTROL

### LARVAE

The efficacy of DDT as a larvicide for the control of *Simulium* was first reported by Fairchild and Barreda (1945) after tests in Guatemala. Swift, mountain streams were treated with emulsions containing DDT, and with cakes of plaster of paris or sawdust impregnated with DDT. Emulsions containing 4 per cent DDT completely eradicated *Simulium* larvae in mountain streams for a distance of 10 kilometers. Steward (1946), Garnham and McMahon (1947), and Prevost (1947) reported further on the early use of DDT for the control of *Simulium*.

The initiation in 1947 of investigations in Canada on the use of chlorinated hydrocarbons and other newer insecticides for the control of immature stages of *Simulium* was described by Hocking, Twinn, and McDuffie (1949). An effective formulation and dosage was developed, i.e., DDT in fuel oil solution, at a concentration of 0.1 p.p.m. for 15 minutes at the point of application, or, as it may be expressed, 1.5 p.p.m.-minutes. Further tests were conducted at Churchill, Man., and Whitehorse, Y.T., in 1948 and 1949, respectively (Hocking, 1950), and at Churchill in 1951 and 1952 (Hocking, 1953). Various formulations of the following insecticides were included: DDT, TDE, toxaphene, chlordane, lindane, heptachlor, dieldrin, aldrin, endrin, isodrin, trichlorobenzene, pyrenone, parathion, malathion, and schradan. Only heptachlor gave satisfactory control of larvae and was a practical substitute for DDT. Some forms of lindane were toxic to pupae but not to a degree sufficient for practical use. The remaining insecticides were not toxic to eggs or pupae.

A solution of DDT in fuel oil was used in 1948 to control the cattle-infesting black fly, *S. arcticum*, in the Saskatchewan River. Arnason *et al.* (1949) reported that a single aerial application of DDT in fuel oil, at 4.68 p.p.m.-minutes, controlled the *Simulium* larvae for a proved distance of 17 miles and a possible distance of 90 miles downstream. These investigations were continued with applications from bridges and ferrys (Fredeen *et al.*, 1953). It was shown that the river could be cleared of larvae as far as 115 miles downstream by the application of 10 per cent solutions of DDT at concentrations as low as 0.9 p.p.m. or 1.4 p.p.m.-minutes. The DDT was found to be associated with suspended solids in the river (Fredeen *et al.* 1953; Berck, 1953). There were 551 parts of solids per million parts of water, and samples taken as far downstream as 68 miles held 0.24 to 2.26  $\mu$ gm. of DDT per gram of solids. *Simulium* larvae ingest the suspended solids and this was suggested as an explanation for the specificity and long range effects of DDT. Fredeen and co-workers have recommended that, in the treatment of clear-water streams, a finely-divided inorganic material with DDT-adsorptive qualities should be mixed with the DDT.

Following the success of the Saskatchewan River treatments, an experiment in area control of black flies was made at Goose Bay, Lab., in 1950. Hocking and Richards (1952) described the treatment of 77 streams and rivers in a 200 square mile area within an 8-mile radius of the airport at Goose Bay. A 10 per cent solution of DDT in fuel oil was applied from a Dakota (C-47) aircraft, from a helicopter, a boat, and from points on the ground. The treatments caused a large reduction in the black-fly infestation at the centre of the treated area, and a noticeable reduction within a radius of 4 to 6 miles. Hocking and Richards concluded that, where *P. hirtipes* is the important species, the larvae should be eliminated

within a radius of 2 miles; whereas a 6-mile radius is necessary for *S. venustum*. Stream treatments with DDT at concentrations of 1.5 p.p.m.-minutes in British Columbia were reported by Curtis (1955).

The method has now been adopted by the Armed Services, the Ontario Hydro Commission, mining companies (Roach, 1954), and other organizations whose interests are in areas infested by black flies. However, individual stream treatments are impractical in many regions of Canada because of rugged terrain, heavy forest cover, absence of roads and trails, or excessive costs in time and labour.

The following discussion concerns studies on control that have been conducted recently near Baie Comeau, Que., on the north shore of the St. Lawrence River. In 1954, the Georges Tremblay River drainage basin, a total area of approximately 26,000 acres, with a perimeter of 42 miles, was treated by aerial application for the control of larvae (Brown, 1955; Peterson, 1955). One thousand and eighty-eight gallons of a 10 per cent solution of DDT in fuel oil was applied to the periphery of the watershed from a DHC-2 Beaver aircraft fitted with a rotary brush spray assembly. The purpose of the experiment was to determine whether sufficient DDT would be washed into the streams within the basin to control the black-fly larvae, and significantly reduce the subsequent infestation of flies. About 58 per cent of the streams within the basin have their sources at or near the periphery, and were possibly affected by the application. The larval population at the sampling points in these streams was reduced by 98 per cent. However, the subsequent adult infestation was not significantly reduced. Large numbers of flies emerged from streams that were unaffected by the treatment, and, later in the season, flies emerged even from some of the treated streams.

The Wood River drainage basin was selected for further tests in 1955. DDT solution was applied from the Beaver aircraft on two plots, in flights across the basin, and along parallel lines at one-quarter mile intervals. One plot, approximately 15 square miles in area, was treated with a 10 per cent solution of DDT in fuel oil for an average dosage of approximately 1/50 of a pound of DDT per acre, on a regional basis. A 15 per cent solution was applied on a second plot, approximately 11 square miles in area, to give an average dosage of approximately 1/10 of a pound of DDT per acre, on a regional basis. Sampling cones were used for assessing larval infestations in streams in the treated plots and in a check plot. The infestations in the latter remained relatively stable while the spray was being applied. Nine streams were assessed in the first plot, of which two contained infestations that were too light for analysis. Four streams were completely cleared of larvae by the treatment, and three were not affected since they lay between the flight lines and parallel to them. The larvae in the streams in the second plot were reduced or eliminated. The application of 1/50 of a pound of DDT per acre appeared as effective as 1/10 of a pound.

Hocking, Twinn, and McDuffie (1949) noted the residual effect of aerial as compared to ground applications. Hocking presented the hypothesis that control of black-fly larvae is not necessary in perma-frost areas where DDT is applied for any purpose at an average rate in pounds per acre per annum that exceeds 0.012 times the annual precipitation. The control obtained in the Adirondacks by Travis and co-workers (1951), who applied an average rate of about 0.0084 pounds of DDT per acre, was cited as an example. Unfortunately, a prolonged drought in the summer of 1955 prevented an assessment of the residual effect of the treatment of the plots in the Wood River drainage basin. In May, 1956, the entire Wood River basin was treated and reports should shortly be available on the immediate and residual effects.

Manipulation of the flow of water in streams to control *Simulium* larvae has been discussed by Dampf (1931). His description of methods of combatting onchocerciasis in Mexico included the periodic deflection of streams, and the construction of numerous dams to convert streams into a system of receptacles of calm water which would cause the death of most species of *Simulium*. The periodic deflection of streams would be impractical in most regions of Canada. However, the authors observed the effects of dams in streams in pulpwood cutting areas in Quebec. The dams facilitate the movement of the pulpwood logs to the mill in the spring of each year. The areas of calm water behind the dams doubtless reduce the breeding areas. However, the dams and their spillways create excellent oviposition sites for many species including the major pest, *S. venustum*. The overall effect would be difficult to assess.



The effect of floods on larval infestations in streams was observed. Many of the streams in a pulpwood cutting limit are flushed each spring to move the logs downstream. The combined effect of the sudden rise in water level and the constant disturbance of the stream bed caused by the logs eliminated black-fly larvae. Larvae reappeared in the streams when logging ceased, but at least two years were required to restore the larval population to its former level. Flooding, following heavy rain or the opening of a dam and without the passage of logs, washed many larvae downstream into calm sections where the rate of survival is low. Unfortunately, the use of flooding as a control method in pulpwood cutting areas is prevented by practical difficulties.

#### ADULTS

The control of black-fly adults by an aerial application of DDT was demonstrated in 1954 at Franquelin, Que. (Peterson, 1955). The procedure was based on the successful control of adult mosquitoes by aerial applications of DDT at a dosage of 1/4 of a pound per acre, over a minimum area of 10 square miles to prevent rapid re-infestation (Goldsmith *et al.*, 1949; Brown, 1951, 1952; Sharp, 1952). The effect of such a treatment on black flies had not previously been assessed in Canada. The Beaver aircraft was used to treat an area of 6.7 square miles with a 10 per cent solution of DDT in fuel oil. The aircraft flew parallel flight lines at 200-yard intervals and a dosage of 0.17 pounds of DDT per acre was applied. The number of black flies in the treated area, as compared to the number in the untreated surrounding area, was reduced by the application. The experiment was repeated in 1956 to confirm these results.

#### ACKNOWLEDGEMENTS

The authors are grateful to Dr. C. R. Twinn, Head, and Mr. F. J. H. Fredeen, Associate Entomologist, Veterinary and Medical Entomology Unit, Canada Department of Agriculture, as well as to Prof. F. P. Ide, University of Toronto, and Prof. D. M. Davies, McMaster University, for permission to refer to unpublished data. The helpful criticism of Dr. Twinn, Mr. Fredeen, Prof. Davies, and of Prof. A. S. West, Queen's University, and Prof. B. Hocking, University of Alberta; is gratefully acknowledged.

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## APPENDIX

## A LIST OF THE SPECIES OF SIMULIIDAE RECORDED IN CANADA

<i>Prosimulium decemarticulatum</i> (Twinn)	<i>Cnephia borealis</i> (Malloch)
" <i>dicentum</i> Dyar and Shannon	" <i>dacontensis</i> (Dyar and Shannon)
" <i>dicum</i> Dyar and Shanno	" <i>emergens</i> Stone
" <i>fulvum</i> (Coquillett)	" <i>eremites</i> Shewell
" <i>gibsoni</i> (Twinn)	" <i>invenusta</i> (Walker)
" <i>hirtipes</i> (Fries)	" <i>minus</i> (Dyar and Shannon)
" <i>multidentatum</i> (Twinn)	" <i>mutata</i> (Malloch)
" <i>pleural</i> Malloch	" <i>saileri</i> Stone
" <i>ursinum</i> (Edwards)	" <i>sommermanae</i> Stone
" <i>vernale</i> Shewell	" <i>taeniatifrons</i> (Enderlein)
<i>Simulium</i> ( <i>Eusimulium</i> ) <i>aureum</i> Fries	<i>Simulium</i> ( <i>Simulium</i> ) <i>arcticum</i> Malloch
" " <i>baffinense</i> Twinn	" " <i>bivittatum</i> Malloch
" " <i>canonicolum</i> (Dyar and Shannon)	" " <i>canadense</i> Hearle
" " <i>clarum</i> Dyar and Shannon	" " <i>corbis</i> Twinn
" " <i>croxtoni</i> Nicholson and Mickel	" " <i>decorum</i> Walker
" " <i>euryadminiculum</i> Davies	" " <i>fibrinflatum</i> Twinn
" " <i>furculatum</i> Shewell	" " <i>griseum</i> Coquillett
" " <i>gouldingi</i> Stone	" " <i>hunteri</i> Malloch
" " <i>innocens</i> Shewell	" " <i>jenningsi</i> Malloch
" " <i>johannseni</i> Hart	" " <i>kamloopsi</i> Hearle
" " <i>latipes</i> (Meigen)	" " <i>luggeri</i> Nicholson and Mickel
" " <i>pugetense</i> (Dyar and Shannon)	" " <i>malyshevi</i> Dorogostajakij, Rubzov and Vlasenko
" " <i>rivuli</i> Twinn	" " <i>meridionale</i> Riley
" " <i>subexcisum</i> Edwards	" " <i>nigrocoxum</i> Stone
<i>Simulium</i> ( <i>Neosimulium</i> ) <i>vittatum</i> Zetterstedt	" " <i>parnassum</i> Malloch
<i>Twinnia tibblesi</i> Stone and Jamnback	" " <i>pictipes</i> Hagen
<i>Gymnopaia holoptictus</i> Stone	" " <i>rugglesi</i> Nicholson and Mickel
	" " <i>sayi</i> Dyar and Shannon
	" " <i>transiens</i> Rubzov
	" " <i>tuberosum</i> (Lundstroem)
	" " <i>venustum</i> Say

# Ecological Studies on British Simuliidae

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## ABSTRACT<sup>2</sup>

This is a summary of studies made on the age of females of the black-fly, *Simulium ornatum* Mg., attracted to grazing cattle. A preliminary note has been published (Davies, 1955), and the full results will appear elsewhere.

Dissection of laboratory-kept flies showed that the abundant fat-body present on emergence from the pupa was gradually depleted over 14 days in non-engorged individuals. In females engorged on cow blood, ripe eggs were produced after 4 to 5 days at 16 to 18°C., by which time no detectable fat-body remained in the anterior region of the abdomen. Dissection of flies taken on cattle showed that 90% or more could be ascribed to one of two categories, namely, those containing visible anterior abdominal fat-body and considered to be young and non-blood-fed in view of the results obtained in the laboratory; those containing no fat-body in that region and considered to be mainly old individuals which had obtained a previous blood-meal and had undergone a complete gonotrophic cycle. Certain individuals, of the latter category only, contained relict ripe eggs. The absence of relict eggs in field flies containing visible fat-body shows that *S. ornatum* females are normally unable to rebuild the fat-body reserves once these have been depleted.

Using the above system of approximate age-classification, it was found that during the middle of the season (July to late Aug., 1954), the proportion of old flies among the total flies landing on cattle tended to be inversely correlated with fly abundance, as would be expected on theoretical grounds. Towards the close of the season (Sept.), however, both fly abundance and the proportion of old flies increased. This suggests that fly longevity was greater towards the end of the season than in mid summer.

On days in mid-season flies classed as old were normally taken on the cattle in numbers only in late evening, whereas the majority of flies active during late morning and afternoon were young individuals. In contrast, on days in early autumn, old flies landed on the cattle in numbers throughout the day. By a study of events on certain mid-season days when the numbers of old flies failed to show the normal increase in late evening, evidence was obtained that curtailment of total fly activity at the end of the day by unusually rapid fall in air temperature, had led to the omission of the phase characterized by the abundance of old flies. This suggests that the activity of old flies may be triggered off by such a factor as falling light intensity. Thus if the air temperature falls rapidly in early evening, by the time that light intensity is at the correct level or rate of fall to stimulate the activity of old flies, air temperature may be too low for flies to be active so that no increase in old fly numbers occurs.

Comparison of the percentage reduction in the numbers of old and young flies on windy evenings as compared with their numbers on adjacent calm evenings, showed that old flies suffered about twice as great a percentage reduction in numbers as did young flies. This suggests that older individuals were more adversely affected by wind, possibly through a lowered desiccation resistance or lower energy available for flight.

The normally greater number of old flies active in late evening, as compared with during the day, shows that in the case of a disease organism transmitted by *S. ornatum*, a susceptible host would often be exposed to a greater number of potentially infective bites in late evening than during the day.

## REFERENCE

Davies, L. 1955. Behaviour of young and old females of the black-fly, *Simulium ornatum* Mg. *Nature* 176: 979-980.

## DISCUSSION

R. LEVI-CASTILLO. Regarding the determination of age in the *Simulium* population, how do you actually know which are young and which are old forms?

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<sup>2</sup>A full account has appeared in the *Bull. Ent. Res.* 48: 535-552. 1957.

L. DAVIES. By rearing adults from pupae and keeping them in the laboratory and studying the disappearance of fat body in them. Also by studying the complete absence of fat body in flies fed on cow blood, by the time they became fully gravid.

J. A. DOWNES. Is the egg-laying period of *S. ornatum* rather sharply defined and in the late evening? In *Culicoides nubeculosus*, females fly (for hunting) both morning and evening; the egg-laying flight is evening only, and, in laboratory experiments at least they bite readily immediately after oviposition. Could your evening peak of 'old' biters be due to immediately preceding egg-laying, followed urgently by hunting?

L. DAVIES. I think that this may well be the case.

# Virus-Vector-Host Relationships of the American Arthropod-Borne Encephalitides

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## ABSTRACT

*Knowledge of the reactions of viruses in mosquitoes, of viruses in vertebrate hosts, and inter-relationships between mosquito and vertebrate hosts in virus propagation is necessary in an understanding of the epidemiology of mosquito-borne diseases. Of special interest are quantitative relationships between vertebrate viremia and susceptibility of mosquito vectors, quantitative differences in the transmitting efficiency of various mosquito species, factors influencing extrinsic incubation, and rates of virus development within mosquitoes.*

The arthropod-borne encephalitides, as a group of viruses, are especially interesting because of their vector-host relationships. One generally thinks of viruses as being host specific, but these are capable of infecting both vertebrates and invertebrates. A study of the properties of these viruses and of the interrelationships between the viruses and their hosts gives an insight into the complex factors influencing perpetuation of these disease-producing agents.

In this report, the viruses of eastern and western equine encephalitis (EEE and WEE) and of St. Louis encephalitis (SLE) will be considered. It is felt that findings concerning these three American arthropod-borne encephalitides will apply more or less closely to allied viruses found in other parts of the world, and that the principles determining infection and transmission are broad and not restricted to any one disease agent.

Mosquitoes undoubtedly are the principal invertebrate hosts of these agents and serve as primary vectors. The basic transmission cycle appears to be a simple one. It consists of infected mosquitoes feeding upon susceptible vertebrates, particularly birds, which respond by circulating virus in their blood for variable periods. During feeding, more mosquitoes take up this virus and become infected and capable of transmitting it to more birds.

The life-cycle, as here stated, is obviously oversimplified. There are many contributing factors involved which are not understood. For instance, the overwintering mechanism of the viruses in temperate regions cannot be explained. In spite of such gaps in knowledge, however, the epidemiology has been somewhat clarified in the last few years and the endemic and epidemic patterns of infections are beginning to be more clearly seen.

Two types of virus transmission systems appear to be involved in the epidemiology of EEE. One is a low-level, maintenance cycle which seems to account for the endemicity of infection within an area. In it, apparently, the number of mosquito species involved are restricted and their populations need not be above normal levels. These mosquitoes presumably show a distinct preference for the blood of avian hosts and virus transmission can go on year after year at a low rate. It is not likely that man or horses will be bitten by these infected, primarily bird-feeding mosquitoes.

The other transmission system is the epidemic cycle in which the virus infection may go beyond its normal mosquito and bird hosts. An exceptionally high population of a predominant, susceptible mosquito species and a low level of immunity in the general bird population are abetting factors. With the high mosquito numbers, feeding specificity becomes a less important factor, the vertebrate host range is extended, and man and horses may become involved. Such outbreaks, fortunately, are self-limiting, as they come to a halt with the decline of the mosquito population and the development of a high percentage of immunes in the general bird population.

The epidemiological pattern of WEE is similar to that of EEE but differs in one important respect: *Culex tarsalis* is apparently both the endemic and epidemic vector in the western part of the United States. Its feeding habits, with preference for avian blood but a liking for that of mammals as well, suit it admirably for this dual role. In the eastern part of the United States, however, WEE occurs endemically in the absence of *C. tarsalis* and seems to follow the EEE epidemiologic pattern.

The virus of SLE in the western part of the United States appears to be primarily *C. tarsalis* transmitted, and in those regions the epidemiology is probably similar to that of WEE. In the eastern part of the country the *C. tarsalis* population becomes sparser, however, and the major part of the epidemic transmission is taken over by *C. pipiens* and *C. quinquefasciatus*. The role of these two species in endemic transmission is uncertain at the present time.

The remainder of this report is concerned with more detailed studies on the reaction of these viruses within mosquitoes and factors influencing mosquito infection, rather than with broad epidemiological aspects.

#### THRESHOLDS OF INFECTION

The natural means by which a mosquito becomes infected is by ingesting the infected blood of vertebrates during a state of viremia. Quantitative studies revealed, however, that a level of virus infective for one species of mosquito was not necessarily infective for another (Chamberlain *et al.*, 1954b). Some species required only low concentrations of virus to become infected, while others required much higher concentrations. Concurrently, a study was carried out to determine the course of virus infections in various vertebrates (Kissling *et al.*, 1954a, b). Great differences were revealed in the amounts of virus circulating in their blood at the time of infection. Drawing upon the results of these mosquito and host studies, it is obvious, other factors being equal, that the more susceptible mosquitoes potentially would have the greatest opportunity for infection in nature, and that the vertebrates circulating the greatest amounts of virus could serve as the most efficient infection sources. Thus, by the use of laboratory procedures only, it has been possible to single out the potentially more important vectors for further study and surveillance, and to dismiss other species from consideration as being less likely vectors. Similarly, certain bird species were found to possess a very high mosquito-infecting potential, while other vertebrates, such as horses, were shown to be unlikely sources of mosquito infection.

#### INFECTION AND TRANSMISSION RATES

Even when mosquitoes are given a virus meal of high concentration, well above their infection threshold, it is found that some species are more susceptible than others. This is reflected in a higher percentage becoming infected. Also, if individuals of different species are tested for their ability to transmit by bite (after a suitable incubation period) it can be shown that a higher percentage of individuals of some species can transmit than others (Chamberlain *et al.*, 1954b; Chamberlain and Sudia, 1956). Examples for EEE and WEE are shown in Tables I and II. Such quantitative tests provide additional means of assessing the vector potential of various mosquito species. Thus efficient vectors can be separated from the inefficient, and the intermediates are revealed so that they may be judged further for possible importance under particular field conditions.

TABLE I—EEE Infection and Transmission Rates in Mosquitoes.

Species	Per cent infected	Per cent transmitting
<i>Aedes triseriatus</i>	100	86
<i>Aedes sollicitans</i>	100	75
<i>Culex tarsalis</i>	100	65
<i>Aedes aegypti</i>	100	56
<i>Wyeomyia mitchellii</i>	80	50
<i>Culex restuans</i>	45	33
<i>Aedes atropalpus</i>	100	25
<i>Orthopodomyia signifera</i>	76	25
<i>Psorophora confinnis</i>	100	22
<i>Mansonia perturbans</i>	94	20
<i>Psorophora ciliata</i>	83	18
<i>Psorophora ferox</i>	100	15
<i>Culex erraticus</i>	43	14
<i>Aedes vexans</i>	63	13
<i>Anopheles quadrimaculatus</i>	79	0
<i>Anopheles crucians</i>	17	0
<i>Culex quinquefasciatus</i>	5	0
<i>Culex salinarius</i>	3	0



TABLE II—WEE Infection and Transmission Rates in Mosquitoes.

Species	Per cent infected	Per cent transmitting
<i>Psorophora discolor</i>	100	89
<i>Culex tarsalis</i>	93	86
<i>Aedes aegypti</i>	100	84
<i>Aedes triseriatus</i>	100	82
<i>Wyeomyia mitchellii</i> *	100	60
<i>Aedes atropalpus</i>	100	58
<i>Orthopodomyia signifera</i>	100	50
<i>Psorophora confinnis</i>	97	40
<i>Psorophora ferox</i>	100	40
<i>Aedes sollicitans</i>	96	39
<i>Psorophora ciliata</i>	90	30
<i>Aedes vexans</i>	36	29
<i>Culex quinquefasciatus</i>	9	4
<i>Anopheles quadrimaculatus</i>	33	2
<i>Culex salinarius</i>	0	0

\*possibly mixed with *W. vanduzeei*

VIRUS MULTIPLICATION IN MOSQUITOES

Virus multiplication occurs in the mosquito as a result of taking a virus meal. This is difficult to prove if the titer of the infecting meal were high, as it may not be possible to recover more virus from the mosquito than was ingested. However, a study of the amounts of virus in individual mosquitoes at different periods of incubation reveals an initial virus decline, followed by an increase, which suggests that multiplication actually does occur (Chamberlain *et al.*, 1954a).

More direct proof of virus multiplication can be obtained by testing mosquitoes infected from ingesting relatively small amounts of virus, near their threshold levels. Substantial increases in virus concentrations over that ingested can then be demonstrated. For example, a million-fold increase of St. Louis encephalitis virus in *C. tarsalis* has been shown (Chamberlain *et al.*, 1957). A 16,000-fold increase of western equine encephalitis virus has been revealed in this same species and a 6,000-fold increase of eastern equine encephalitis virus in *Aedes sollicitans* (Chamberlain and Sudia, 1956).

THE EXTRINSIC INCUBATION PERIOD

The extrinsic incubation period in a mosquito is that interval between ingestion of an infected blood meal and the time when transmission by bite becomes possible. During this interim, the virus presumably infects cells of the digestive tract, multiplies and is released into the coelomic cavity to spread and infect cells in the rest of the body. Then virus either accumulates in, or multiplies in, the salivary glands. When the concentration in the glands is adequate, infectious concentrations of virus particles are injected with salivary secretions into a host at the time of feeding.

Factors which influence this incubation period are of interest because they affect the virus development in the mosquito. The incubation temperature is an important influencing factor. The lower the temperature, the longer the incubation required, and the higher the temperature, the shorter the incubation period.

Incubation studies which covered the range of temperatures from 70° to 90° F. were conducted with eastern equine encephalitis in *Aedes triseriatus* (Chamberlain and Sudia, 1955). The percentage of mosquito specimens that eventually became capable of transmitting by bite was found to be about the same (near 80 per cent) regardless of the incubation temperature. The time required to reach these transmission peaks differed considerably, however. At 70° F., 34 days of incubation were required, but at 80° only 16 days, and at 90° 10 to 12 days. A combination of temperatures (4 hours daily at 90° and 20 hours daily at 70°) gave results similar to those of a constant 80°.

The incubation study indicated that mechanical transmission can occur early in the incubation period. It was especially evident when the temperature was low, more suitable for virus survival upon the mosquitoes' mouthparts. The mechanical transmission was accomplished with decreasing efficiency for a week by mosquitoes held at 70° F., but only for

3 to 4 days by those at 80°, and for less than 3 days by those at 90°. Following the loss of this initial transmitting ability, the mosquitoes again became capable of transmitting, this time presumably through infection of the salivary glands.

Related studies were performed to determine whether similar results could be obtained by jabbing susceptible host animals with virus contaminated pins after various waiting periods. The findings were similar to those obtained during the early incubation in the mosquitoes and supported the view that mosquitoes can transmit by means of contaminated mouthparts (Chamberlain and Sudia, 1956).

While the ability of mosquitoes to transmit encephalitis mechanically may be of doubtful significance, situations could exist in nature where infection might be more quickly spread if mosquitoes were disturbed in the act of feeding. Examples are the feeding upon an infected bird in a nest of non-infected birds, or upon a horse with an exceptionally high viremia pastured with normal horses.

The extrinsic incubation time can be influenced by differences in physiology of the mosquito species themselves, as well as by temperature. One of the most striking examples of this was observed with St. Louis encephalitis in *Culex quinquefasciatus* and *C. pipiens* (Chamberlain and Sudia, 1956). The *C. pipiens* attained peak transmission rates of 100 per cent within 12 days at 80° F., while the *C. quinquefasciatus* required 22 to 26 days. Another good example, for western equine encephalitis, is found with *C. tarsalis* and *A. aegypti*. The *C. tarsalis* required between 2 and 3 weeks to attain a peak transmission rate of 86 per cent, whereas the *A. aegypti* had reached a peak of 84 per cent within 5 days.

It is not known where the delaying factor lies in those mosquitoes that require longer incubation, nor why virus appears in the salivary glands in high concentration so quickly in the others. It is worth noting, however, that the total virus concentration in the bodies of the fast species is usually greater than that of the slow species. Possibly salivary glands, like cells of the alimentary tract, have their threshold of infection, and their exposure to higher concentrations of virus in the haemolymph may bring about a high level of infection in these organs in a briefer period of time.

#### INFECTION OF MOSQUITOES BY INTRATHORACIC INOCULATION

Until now this report has given consideration only to infections induced in mosquitoes by the natural route, i.e., by ingestion of infected blood. There is another technique, however, which is excellent for carrying out very closely controlled mosquito infection experiments. This technique, developed and used by Dr. Loring Whitman (personal communication), is mosquito inoculation by the intrathoracic route. By this means the alimentary tract can be by-passed and information obtained on the location of infection barriers within the mosquito.

By inoculation into the mosquito, very small amounts of virus have been found adequate for initiating infection (Chamberlain and Sudia, 1956). For example, only 0.1 of a mouse infective unit of WEE virus is adequate to infect *C. tarsalis* or *Aedes triseriatus*. SLE virus is likewise as infectious for *C. tarsalis* and *C. quinquefasciatus*. In these instances the inoculated infective dose possibly may comprise a single virus particle.

Mosquito infection thresholds upon ingestion of virus are usually much higher. In the most susceptible species and most successful vectors, the disparity between the inoculated infective dose and the ingested infective dose may not be great, but in the less susceptible species 10,000 to 1,000,000 times as much virus may be required for infection by the alimentary route. Even some mosquitoes which are almost totally refractory to infection by feeding (e.g. *C. quinquefasciatus* to EEE and WEE (Chamberlain *et al.*, 1954b), *Anopheles annulipes* to Murray Valley encephalitis (McLean, 1955)) may be infected readily by intrathoracic inoculation. These results indicate that the gut presents a natural chemical or physical barrier to infection which limits mosquito susceptibility.

In addition to the gut barrier, McLean has found that salivary glands of *An. annulipes* comprise a second barrier to infection, and subsequent transmission, for although infection of the body with Murray Valley encephalitis virus could be established by inoculation, the specimens remained unable to transmit by bite. Undoubtedly many other aspects of virus infection in arthropods can be revealed by varied application of the intrathoracic inoculation technique.

The work discussed is representative of the type currently being done in pursuance of a better understanding of virus-vector-host relationships. It is highly probable that elucidation of the course of infection within mosquitoes will extend the knowledge on virus propagation in general. Still unknown are the mechanism of virus overwintering and the ecological factors involved in bringing about a change from an endemic to an epidemic state of infection. The solving of these problems for any one of the viruses in the mosquito-borne group is likely to indicate the pattern for the others and lead to more practical means of prevention of disease due to these agents.

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## DISCUSSION

L. M. BLACK. What is the serological relationship between WEE and EEE viruses?

ROY W. CHAMBERLAIN. They are related viruses, in the same group, but fairly serologically distinct. There may be a small degree of crossing in neutralization tests if hyperimmune sera are used. There is also some crossing in hemagglutination tests, but it is not great.

C. B. PHILIP. I judge that you feel that the salivary glands provide a barrier to virus in some mosquito species which are poor or refractory vectors compared to efficient ones.

ROY W. CHAMBERLAIN. Yes, I believe this is the case, as proven both by experiments in which virus was given mosquitoes by ingestion and by inoculation. *Anopheles quadrimaculatus* and *crucians* can become infected (i.e., develop virus in their bodies) following ingestion but cannot transmit. Their barrier, therefore, is not in the gut, but presumably in the salivary glands. By the intrathoracic inoculation technique, McLean has shown *Anopheles annulipes* incapable of transmitting, although virus developed in the body. Again this indicates refractory salivary glands.

W. H. R. LUMSDEN. With regard to the interepidemic survival of yellow fever virus in East Africa, recent laboratory work on mites ectoparasites on *Galago* spp. (which appear to be the mammals involved in the persistence of virus in areas subject to vigorous dry seasons as far as neutralization tests show) do not appear encouraging. Although virus may be reclaimed by inoculation of 3-day mice 4 days after the infecting meal, it was not reclaimed at 16 or 26 days by the same methods. But the distribution of immunity in the *Galago* population indicates that the infection is correlated with the grouping of animals inhabiting a nest.

C. B. PHILIP. It is of interest that there was apparent mechanical transmission after 3 days at low storage temperatures of mosquitoes infected with EEE virus, since I was never able to get transmissions even after immediate interrupted feedings in yellow fever infected *A. aegypti*. The incubation data also remind one that the extrinsic incubation in yellow fever is lengthened not only by lowered temperatures, but by feeding on donors with lower levels of circulating virus.

T. H. G. AITKEN. Apropos of your (Dr. Chamberlain's) remarks regarding resistance of certain species of mosquitoes to virus acquired by feeding and the susceptibility of the same species of mosquitoes when inoculated with virus, I might point out that Dr. Whitman (Rockefeller Foundation Labs., New York) has shown that *Culex fatigans*, which is resistant

to yellow fever virus in feeding experiments, is also resistant to yellow fever virus when it is inoculated.

R. LEVI-CASTILLO. Have you observed the same picture in Venezuelan encephalomyelitis? And what are your observations on South American strains and their high virulence?

ROY W. CHAMBERLAIN. Studies which we have done with Venezuelan encephalomyelitis virus indicate that the reactions in mosquitoes are similar to those for eastern equine encephalitis virus. We have not tested the virulent South American strains you mention.



# Entomological Aspects of the Trinidad Virus Research Program<sup>1</sup>

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## ABSTRACT

*The Trinidad Regional Virus Laboratory was established early in 1953 as a joint undertaking of the Government of Trinidad and Tobago, the Colonial Medical Research Committee, and The Rockefeller Foundation with intent to study neotropical virus diseases in vertebrate hosts and arthropod vectors. Entomological activities are concerned with the taxonomy, biology, and ecology of the island's haematophagous arthropod fauna, and experiments to elucidate the vectorial capacities of suspect species. Methods are described for collecting and processing arthropods for virus studies, and a résumé is presented of accomplishments to date.*

The Trinidad Regional Virus Laboratory was established early in 1953 as a joint undertaking of the Government of Trinidad and Tobago, the Colonial Medical Research Committee, and The Rockefeller Foundation with intent to study neotropical-occurring virus diseases in vertebrate hosts and arthropod vectors.

Blood surveys conducted from the laboratory during 1953 and early 1954 in representative areas of the island, indicated the presence (by neutralization tests) of at least the following six arthropod-transmitted virus infections: dengue, yellow fever, Ilhéus, St. Louis, Venezuelan equine encephalitis, and eastern equine encephalitis. These findings, in the case of the first four, have been substantiated by the isolation of virus on one or more occasions during the past three years. From the evidence, the Venezuelan infection has not been active in the population since 1943, at which time there was a recorded epidemic affecting livestock and human beings (Gilyard 1944, 1945), and the presence of eastern equine encephalitis rests on two unequivocally positive sera from a native donkey (Downs *et al.*, 1955; Anderson *et al.*, 1956; Anderson *et al.*, 1956; Downs *et al.*, 1956).

In addition to determining over-all occurrence, distribution, and importance of arthropod-transmitted virus infections in Trinidad and Tobago, a major objective of the laboratory's program has been an attempt to elucidate the epidemiology of Ilhéus virus. Thus far little progress has been made; however, it appears to be an infection particularly prevalent in young males in the high-rainfall, cocoa-growing areas of the island. Much of our work therefore has been centered in such an area in the northeastern corner of Trinidad.

Entomological activities have been mainly concerned with (1) classification and biological studies of the island's haematophagous arthropod fauna, and (2) systematic collection of arthropods (principally mosquitoes) in selected localities and processing of them for virological studies. There has been a gradual shifting of emphasis reflecting what is hoped will be increasing attention on establishing laboratory colonies of selected arthropods and undertaking experiments to elucidate the vectorial capacities of suspect species. Within recent months, planned investigations have been started in these fields.

## CLASSIFICATION AND BIOLOGICAL STUDIES

Considerable time has been devoted to this phase of the work in order to build up representative collections of the various arthropod groups of interest to us, so that we know what we are working with. There are few treatises pertinent to the Trinidad fauna and the literature is scattered. Reliance has been placed on various specialists to help "put our house in order" and we take this opportunity to acknowledge publicly their assistance. These specialists are: Edward W. Baker (mites), Joseph R. Bequaert (Pupipara), James M. Brennan (trombiculids), Graham B. Fairchild (*Phlebotomus* and tabanids), William L. Jellison (fleas), Phyllis T. Johnson (fleas), Glen M. Kohls (ticks), John Lane (mosquitoes), Carl F. W. Muesebeck (lice), Alan Stone (simuliids), Russell W. Strandtmann (mites), Rupert L. Wenzel (Pupipara), and Willis W. Wirth (heleids). We are likewise indebted to Lyman B. Smith for assistance in bromeliad classification.

<sup>1</sup> The studies and observations on which this paper is based were conducted with the support and under the auspices of the Government of Trinidad and Tobago, the Colonial Medical Research Committee, and The Rockefeller Foundation.

<sup>2</sup> Staff member, The Rockefeller Foundation (Trinidad Regional Virus Laboratory).



Trinidad is a small island of 1,863 square miles situated 10° north of the equator (Census Album, 1948). Geologically it is a part of the South American continent, lying at the mouth of the Orinoco and separated through natural causes by the Gulf of Paria. As would be expected, its faunistic and floristic affinities are with the mainland. The climate is tropical with average annual maximum and minimum temperatures of 84°F and 74°F respectively. Rainfall generally varies from less than 50 inches on the western littoral to 80–120 inches on the east coast and 150 inches or more in the Northern Range. The major portion falls during the seven-month period June to January. With this abundance of water, albeit seasonal, aquatic insects constitute an important part of the fauna.

Mosquitoes have occupied our greatest attention because they are the island's largest group of blood-sucking arthropods. Approximately 124 species, representing 16 genera, are known from the island (based on the literature and our own collecting). Of this number, we have thus far recovered 105 species, about 84% of the total (Table I), and about 30 of these represent new records for Trinidad.

TABLE I—Trinidad Mosquitoes, and Proportion Collected by the Virus Laboratory.

Genus	Number of Species		Genus	Number of Species	
	Known	Collected		Known	Collected
<i>Toxorhynchites</i>	4	4	<i>Aedes</i>	13	11
<i>Anopheles</i>	14	11	<i>Psorophora</i>	6	6
<i>Culex</i>	36	27	<i>Haemagogus</i>	2	2
<i>Deinocerites</i>	1	1	<i>Trichoprosopon</i>	5	4
<i>Uranotaenia</i>	8	6	<i>Wyeomyia</i>	18	17
<i>Aedomyia</i>	1	0	<i>Phoniomyia</i>	5	5
<i>Mansonia</i>	4	4	<i>Limatus</i>	2	2
<i>Orthopodomyia</i>	1	1	<i>Sabethes</i>	4	4
TOTAL				124	105

The richest genera are *Culex*, *Wyeomyia*, *Anopheles* and *Aedes* with 36, 18, 14 and 13 species respectively. From the standpoint of general mosquito prevalence, however, the most commonly encountered species in forested areas are frequently the sabethines, characteristic mosquitoes of neotropical jungles. Representing as much as 85% of the catch (human bait) at times, they are largely made up of *Wyeomyias*, but all of the genera may be amply represented save for *Sabethes*. Where the terrain is flat, the rainy season sees the ground-pool breeding *Aedes* and *Psorophora* becoming dominant, and species such as *Aedes serratus* (Theobald) and *Psorophora ferox* (Humboldt) are then very abundant. Other commonly encountered species under certain local situations include the bromeliad-breeding *Kerteszi*as, *Anopheles bellator* Dyar and Knab and *A. homunculus* Komp, the coastal *Anopheles aquasalis* Curry, and the swamp-breeding *Mansonia arribalzagai* (Theobald).

Biological studies have included investigations of breeding places, seasonal species prevalence patterns, and vertical stratification of adults in forest habitats. The rainy season obviously is the period of greatest mosquito abundance. With the decrease in rainfall ground pools dry and the forest mosquito population is largely represented by the plant container-breeders which appear to be able to find some water in their micro-habitats for continued but restricted development.

Studies of the arboreal stratification of blood-sucking insects have been carried on by us in three localities, but our remarks will be limited to the Rio Grande Forest tree station inaugurated in October 1955. This station is located in a dense mora forest in northeast Trinidad 1¼ miles from the coast. The land is low-lying and swampy in places. The tree itself is a mora (*Mora excelsa* Benth.) and has four platform levels, at 25, 50, 75 and 95 feet.

We have adopted a means of ascent into the tree differing from the usual system of ladders nailed to the trunk. A tall sturdy pole was sunk firmly in the ground about five feet from the trunk. Rungs bolted to opposite sides of the pole were strung to opposite sides of the trunk and spiked into position, thus forming a series of triangles from pole to trunk up the tree. Three poles, one on top of the other, were used to carry this off-set ladder into the forest canopy. It was simple then to provide platforms at any desired level. Access

to the platforms is through trap doors. Ascent of the ladder is easily made within the triangle, which dispels the feeling of height, and there is no danger of the structure's pulling away from the tree. These more than usual precautions were undertaken because we plan a long-term study in the area.

Thus far, collections at this station have been limited to the morning hours from eight to 12 o'clock (occasionally one o'clock). However, before long we plan to study the mid-day period when arboreal species might be more active. It is hoped to undertake 24-hour studies also. Ground catches have always been made concomitantly with platform catches for comparison.

Collection figures for the period October 1955 through May 1956 at 95 feet and ground level have been summarized (Table II). Of the 28 species selected for study, all refer to mosquitoes except for two species of simuliids and a heleid. While none produced evidence of being strictly arboreal, six showed preference for the forest canopy, particularly *Simulium samboni* Jennings, *Haemagogus s. spegazzinii* Bréthes, *Sabethes chloropterus* (Humboldt),

TABLE II—Vertical Distribution of most Prevalent Diurnal Haematophagous Diptera at Rio Grande Forest Tree Station\*. October 1955 thru May 1956.

Insect	Percent Collected		Number Collected
	Canopy (95')	Ground	
1. <i>Simulium samboni</i>	98	2	58
2. <i>Haemagogus s. spegazzinii</i>	94	6	368
3. <i>Sabethes chloropterus</i>	91	9	57
4. <i>Phoniomyia trinidadensis?</i>	84	16	2,012
5. <i>Anopheles bellator</i>	71	29	51
6. <i>Phoniomyia lassalli</i>	70	30	335
7. <i>Simulium incrustatum</i>	57	43	49
8. <i>Culicoides diabolicus</i>	42	58	80
9. <i>Wyeomyia medioalbipes</i>	9	91	418
10. <i>Trichoprosopon theobaldi</i>	<1	>99	141
11. <i>Limatus durhami</i>	<1	>99	823
12. <i>Aedes scapularis</i>	<1	>99	2,134
13. <i>Mansonia arribalzagai</i>	<1	>99	9,940
14. <i>Psorophora ferox</i>	<1	>99	5,613
15. <i>Psorophora cingulata</i>	<1	>99	95
16. <i>Aedes hortator</i>		100	96
17. <i>Aedes serratus</i>		100	11,557
18. <i>Anopheles aquasalis</i>		100	628
19. <i>Mansonia venezuelensis</i>		100	791
20. <i>Psorophora albipes</i>		100	5,988
21. <i>Psorophora lutzi</i>		100	137
22. <i>Wyeomyia howardi</i>		100	1,794
23. <i>Wyeomyia ypsipola</i>		100	174
24. <i>Phoniomyia splendida</i>		100	57
25. <i>Wyeomyia aporonoma</i>		100	132
26. <i>Mansonia wilsoni</i>		100	415
27. <i>Culex caudelli</i>		100	1,301
28. <i>Limatus flavisetosus</i>		100	2,104
TOTAL			47,348

\*Species with less than 49 specimens not represented.

and the species tentatively considered to be *Phoniomyia trinidadensis* (Theobald). The last is by far the most commonly encountered canopy mosquito. Two species, *Simulium incrustatum* Lutz and *Culicoides diabolicus* Hoffman, appear to be little affected by height and light intensity, being found at all levels quite uniformly. On the other hand, the vast majority of the Rio Grande Forest mosquitoes, such as *Aedes*, *Psorophora* and *Mansonia*, are primarily inhabitants of the ground level area and rarely stray into the upper strata.

Haematophagous diptera other than mosquitoes have occupied much less of our attention. While small numbers of simuliids, *Culicoides* and *Phlebotomus* have been processed for virus isolation, our work with these groups has been largely exploratory, finding

out what species are present and learning something of their feeding habits. Light traps have produced much of the heleid and psychodid material. Thus far, 23 species of *Culicoides* are known from Trinidad and nine of these represent new island records. *Culicoides diabolicus* is by far the most frequent man-biter in forest areas. Nine species of *Phlebotomus* have been recovered; few biting collections have been made, but *Phlebotomus gomezi* Nitzulescu is the most commonly encountered species. Four species of simuliids have been taken by our collectors, of which three quite commonly attack man in forested areas. The Tabanidae are represented by 40 species; we have recovered 23, of which four represent new records for the island.

Large collections of ectoparasites have been amassed mainly as a result of planned programs of small mammal trapping and bird hunting in the search for virus infections in the wild animal population. Approximately 838 mammals, 232 birds and 52 reptiles have been examined through May 1956. While much of the ectoparasitic harvest is still being studied, a fair amount of information is already available. In the haematophagous groups we can report 10 species of ticks, eight species of fleas, five anoplurans, at least 15 species of trombiculids, and seven species of other mites. There have also been a fair number of collections of *Pupipara*, mainly from bats.

#### COLLECTION OF ARTHROPODS FOR VIRUS STUDIES

Emphasis of entomological activities has been placed on sampling the haematophagous arthropod population for naturally-occurring virus. Mosquitoes receive the greatest attention and most of the work has been carried on in forested areas.

During 1953 the processed arthropods largely came from the St. Patrick Estate, 25 miles from Port-of-Spain in the Arima Valley; minor collections were made in scattered areas and at houses of fever suspects. In 1954 the work continued at St. Pats, but two other important sources of mosquitoes included a donkey-baited Shannon Dawn Trap at La Paille Village, which provided vast numbers of *Anopheles aquasalis* from the Laventille Swamp, and a twilight capture from the Arena Forest near San Rafael. When yellow fever appeared on the scene in April, the program was reoriented to investigate the outbreak. Intensive collecting was carried on mainly in three areas where the virus was known to exist: Cumaca, in the Northern Range; Charuma Forest, in the Central Range; and Melajo Forest on the northeast coast. The bulk of the arthropods processed in 1954 came from these three forest areas, with minor collections in scattered localities and around fever houses.

With the disappearance of yellow fever at the end of 1954, laboratory studies were reoriented around a planned investigation of Ilhéus virus in the Melajo Forest and the Sangre Grande area. The Melajo Forest was selected because (1) it was in the general area of Ilhéus infection, (2) it was convenient to Sangre Grande where we established a clinic, (3) the forest was the scene of large-scale lumbering operations and so provided a promising association of humans and mosquitoes, and (4) we still had a tree platform (albeit somewhat rickety) remaining from the previous season's operations. Meanwhile, plans were formulated for the establishment of a tree station in a nearby forest which would not come under the woodsman's axe before 1958 and so would serve as a study area of naturally-occurring virus in the virtual absence of man. The 1955 activities were, therefore, mainly restricted to the Melajo Forest until October, when completion of the new tree station caused a transfer to the Rio Grande Forest, where operations have continued to date. Throughout, we have been prepared to move into any area if the isolation of a human agent made such a move advisable, and this has happened on a number of occasions.

With the exception of the catch from the Shannon trap, all mosquitoes for virus studies have been taken with human bait using five to six boys. Mosquitoes are caught with plastic aspirator tubes and transferred to plaster-of-Paris-lined pint Mason jars fitted with an inverted perforated plastic cone in the screw cap. The plaster-of-Paris not only can be moistened to increase humidity, but it also serves as an excellent resting surface for insects. The jars of mosquitoes are sent to the Port-of-Spain laboratory in portable ice boxes where they are usually stored overnight at 4°C before processing. The living insects are stunned with tobacco smoke prior to identification. As the species are sorted they are kept in separate tubes in an ice bath. Bovalbumin (0.75%) in phosphate-saline buffer fortified with antibiotics is the diluent used and alundum is added to facilitate grinding. Diluent is used at the rate of one c.c. for the first 10 mosquitoes, two c.c.'s from 11 to 300 mosquitoes, and three c.c.'s

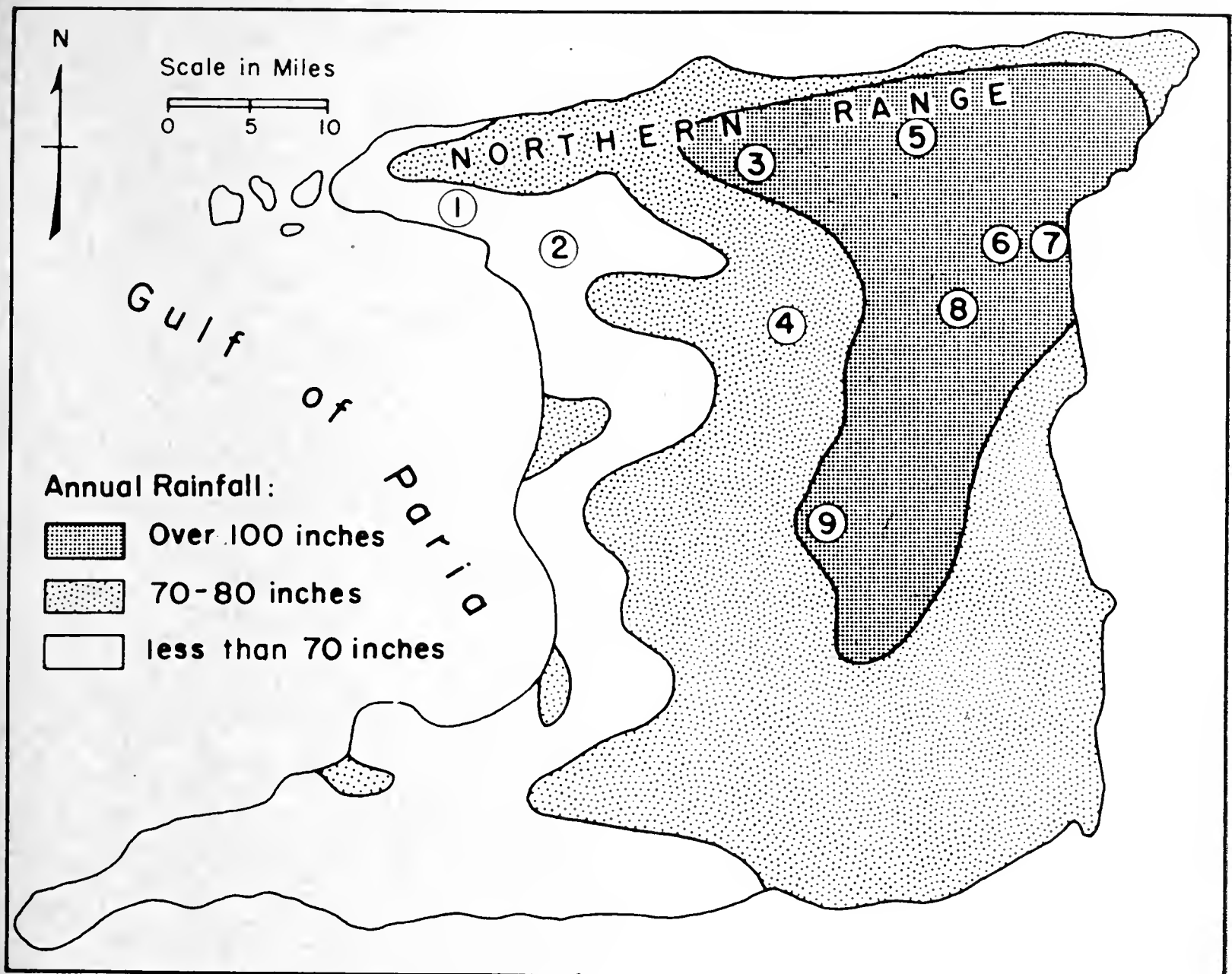


Fig. 1. Trinidad, showing rainfall regions and areas where entomological activities of the virus laboratory were principally carried on: (1) Port-of-Spain virus laboratory, (2) La Paille Village, (3) St. Patrick Estate, (4) Arena Forest, (5) Cumaca, (6) Melajo Forest, (7) Rio Grande Forest, (8) Sangre Grande clinic, (9) Charuma Forest.

for pools above 300. Suspensions are centrifuged at 10,000 r.p.m. for  $\frac{1}{2}$  hour at  $4^{\circ}\text{C}$  and the supernate inoculated intracerebrally into mice. Prior to May 19, 1955, 21-day-old mice were used for all arthropod inoculations; since that time two-day-old mice have been substituted.

From August 21, 1953 through May 1956 approximately 486 thousand arthropods were ground and inoculated into mice in the search for viruses. Of this number approximately 482 thousand represent mosquitoes (Tables III and IV). During the first two years there was a preponderance of sabethines (mainly *Wyeomyias*) inoculated, but during the past two years the culicines have predominated, largely as a result of our operations in the northeastern forests where the terrain favors the ground-pool breeders. Over the past four years, the genera most frequently inoculated have been *Wyeomyia*, *Psorophora*, *Anopheles*, and *Aedes*.

TABLE III—Trinidad Arthropods Ground and Inoculated into Mice (August 1953—May 1956).

Mosquitoes	481,972
Heleids ( <i>Culicoides</i> )	1,518
Simuliids	1,308
Psychodids ( <i>Phlebotomus</i> )	972
Lice	161
Ticks	47
Mites	5
TOTAL	485,983





Fig. 2. Rio Grande Forest tree station, showing 25-foot platform.

TABLE IV—Trinidad Mosquitoes Ground and Inoculated into Mice, by Genus (August 1953—May 1956).

Genera	1953		1954		1955		1956		TOTAL	
	No.	%	No.	%	No.	%	No.	%	No.	%
<i>Aedes</i>	93	1	12,278	5	30,936	19	11,505	31	54,812	11
<i>Anopheles</i>	401	4	58,299	21	6,279	4	675	2	65,654	14
<i>Culex</i>	147	1	2,116	1	7,439	5	379	1	10,081	2
<i>Haemagogus</i>	116	1	6,584	2	1,983	1	309	1	8,992	2
<i>Mansonia</i>	61	<1	3,469	1	4,345	3	2,274	6	10,149	2
<i>Psorophora</i>	1,112	11	11,483	4	51,903	32	8,530	23	73,028	15
<i>Limatus</i>	586	6	4,100	2	22,619	14	2,026	5	29,331	6
<i>Phoniomyia</i>	137	1	6,663	2	7,738	5	9,402	25	23,940	5
<i>Sabathes</i>	871	8	800	<1	99	<1	70	<1	1,840	<1
<i>Trichoprosopon</i>	380	4	13,839	5	2,697	2	88	<1	17,004	4
<i>Wyeomyia</i>	6,532	63	155,037	56	23,332	15	2,240	6	187,141	39
TOTAL	10,436	100	274,668	99	159,370	100	37,498	100	481,972	100

Thus far 61 virus strains have been isolated, all from mosquitoes. While no strains appeared in 1953, 24 showed up in 1954, 32 in 1955, and 5 during the first half of 1956 (Table V). All but two of the 24 strains in 1954 were yellow fever (Downs *et al.*, 1955), the exceptions being Ilhéus virus (Anderson *et al.*, 1956). Not all of the more recent isolations have been positively identified, but the evidence suggests that four are strains of Ilhéus, and three appear to be related to St. Louis virus. Agents have been isolated from



the following genera: *Aedes*, *Anopheles*, *Culex*, *Haemagogus*, *Limatus*, *Psorophora*, *Trichoprosopon*, and *Wyeomyia*. With the exception of one mixed pool, all of the yellow fever strains came from pools of *Haemagogus s. spegazzinii*. The greatest number of virus isolations has consistently turned up during the months of August and September. This is well into the rainy season when forest mosquitoes are most abundant; it is likewise a period when fevers are prevalent.

TABLE V—Virus Strains Isolated from Trinidad Mosquitoes (August 1953—June 1956).

Month	1953	1954	1955	1956	TOTAL
January	—	0	0	2	2
February	—	0	0	0	0
March	—	0	0	0	0
April	—	0	0**	1	1
May	—	0	1	1	2
June	—	0	1	1	2
July	—	0	4	—	4
August	0*	10	13	—	23
September	0	13	10	—	23
October	0	1	1	—	2
November	0	0	0	—	0
December	0	—	2	—	2
TOTAL	0	24	32	5	61

\* Inoculations commenced 21/VIII/53.

\*\*Baby mice (two-day-old) substituted for original mosquito suspension inoculations—19/IV/55.

It is of interest to note that at least 28 of the 37 isolations since April 19, 1955 (when two-day-old mice were substituted for adult mice) are baby mouse agents which would otherwise have been missed in the older mice. One wonders how many more might have been isolated had infant mice been used in the earlier years, particularly in 1954 when over a quarter of a million mosquitoes were tested for virus.

As for the last two spheres of activity mentioned at the beginning of this paper, there is little to report at the present time.

#### INSECT COLONIES

Early in 1954 a colony of local *Aedes aegypti* (Linné) was established. It flourished for about four months and was then destroyed because of the presence of yellow fever in Trinidad. More recently our attention was directed toward *Culex pipiens fatigans* Wiedemann. At the time of writing, the fourth generation had appeared and the colony seems fairly established.

We have also made attempts, without success, to colonize *Aedes ioliota*, Dyar and Knab, *Culex coronator*, Dyar and Knab, and *Psorophora ferox*. The purpose behind these endeavours is, of course, to have quantities of well-nourished uninfected mosquitoes on hand for virus transmission studies.

#### TRANSMISSION STUDIES

Failure to colonize either *Culex coronator* or *Psorophora ferox* has retarded transmission investigations. Both species are of interest to us because of the viruses isolated from them in the wild; a St. Louis-like virus came from *coronator* and Ilhéus from *ferox*.

Pending further studies with these or related species, we have undertaken one experiment with Ilhéus virus (Brazilian 3089 strain) using wild caught *Aedes serratus* and *Psorophora ferox*. While it seems evident that virus was recoverable from triturated mosquitoes 14 to 29 days following an infective meal, there was no transmission by bite.

This then is a very brief résumé of entomological activities at the Trinidad Regional Virus Laboratory. We are not following any rigidly defined plan but orient our work according to the needs of an ever unfolding program.

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## DISCUSSION

R. LEVI-CASTILLO. 1. What is the actual subspecies of *Haemagogus spegazzinii* in Trinidad? 2. Can *H. spegazzinii* be found at ground level? 3. Have you worked the natural breeding places of larvae of phytotelmic Culicidae and recorded them by species?

T. H. G. AITKEN. 1. Thus far we have recognized only typical *spegazzinii*. 2. Yes, the species is found at ground levels as well as in the forest canopy. Under certain conditions, however, it appears to prefer the canopy. 3. Yes, we are correlating adult catches with searches for natural breeding places.

R. LEVI-CASTILLO<sup>3</sup>. I disagree on the presence of the subspecies *H. spegazzinii spegazzinii* in Trinidad, because I have collected personally in that locality and what I found was *H. spegazzinii falco* but never *H. spegazzinii spegazzinii*. Probably no mesosomes were examined in dissected terminaliae of your specimens; otherwise I am sure that what you would have found might have been typical *H. spegazzinii falco*.

T. H. G. AITKEN<sup>3</sup>. We have examined the male terminalia of Trinidadian *Haemagogus spegazzinii* and thus far have seen mesosomes resembling only those of the typical form, or slight variations of this. Because of the known variability of mesosome structure existent in the *spegazzinii* population in certain parts of its range between Brazil and Colombia, it seems quite reasonable to assume that it may likewise occur in Trinidad. Under the circumstances, it might be wisest to eliminate the use of the trinomial for our insular form.

C. B. PHILIP. How recently have yellow fever strains been isolated in Trinidad?

T. H. G. AITKEN. The last isolation of virus was made from a dead red howler monkey coming from the Nariva Swamp (Plum Mitán area) in January 1955.

R. GEIGY. 1. In catching presumably yellow fever vectors, did you use only human baits or also light? 2. Could you localize certain mosquito species on special levels or were they found on the ground as well as at 95 feet? 3. Could you find infected mosquitoes at all levels?

T. H. G. AITKEN. 1. Human bait catches only were attempted. 2. Mosquito species showed preference for certain levels but could be found at others. This was less true of the ground-inhabiting forms which showed less inclination to go up into the higher positions of the canopy. 3. Yellow fever-infected mosquitoes were found at ground level as well as in the canopy.

# Animal Viruses in Arthropods in Canada - The Known and the Unknown

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## ABSTRACT

This paper reviews the limited knowledge of arthropod-borne viruses and their associated vectors in Canada. Western equine encephalomyelitis has been the only virus disease of importance. The agent of Colorado tick fever has been isolated from ticks but no human cases have been recognized. A major portion of the paper considers the possibility that virus diseases, and particularly the arthropod-borne encephalitides, might become more important in the future. The unknown potential vector-ability of numerous species of biting flies, the possible existence of unrecognized viruses, the evidence of recent introductions of potential vectors, the possibility of new introductions of viruses or of vectors, and factors favouring the increase of indigenous known or potential vector species are considered.

## INTRODUCTION

In common with other cold temperate regions Canada is usually correctly regarded as being relatively free from arthropod-borne diseases. However, exceptions to this generalization do occur, one of the more striking being the epidemic of malaria at Archangel, north of the arctic circle, at the end of World War I. The at least sporadic occurrence of an arthropod-borne disease cannot be ruled out north of some arbitrarily selected line.

While it is admitted that no major epidemics of arthropod-borne diseases have been recorded in Canada, an imposing number of such diseases have been recognized at one time or another. Included are: malaria, epidemic typhus, sylvatic plague, relapsing fever, Rocky Mountain spotted fever, tularemia, Colorado tick fever, and western equine encephalomyelitis.

The last two of the foregoing list are the only arthropod-borne virus diseases which have been detected in Canada and of these only western equine encephalomyelitis (WEE) can be regarded as of significance<sup>1</sup>. However, with a rapidly increasing population, with the opening up of formerly isolated areas in many parts of which biting flies are present in hordes, and in view of the ever increasing knowledge of arthropod-borne viruses in many parts of the world, a consideration of the potential of this group of diseases in Canada is warranted. It should be borne in mind that the importance of a disease such as WEE is not in terms of numbers. As Beadle<sup>2</sup> (1952) points out with respect to eastern equine encephalomyelitis (EEE), "The importance lies in hysteria or panic which accompanies the unpredictable outbreaks. People are stricken with fear, and consequently publicity is tremendous".

## COLORADO TICK FEVER

In North America Colorado tick fever is apparently becoming of increasing importance. It is no longer regarded as a benign disease. Improvements in diagnostic and clinical facilities in the United States have shown that the virus is more pathogenic than formerly considered. The distribution of the disease closely coincides with that of its principal vector, the tick *Dermacentor andersoni* Stiles. *D. variabilis* (Say) is also a vector. Insofar as is known no human cases have been reported in Canada. However, both species of ticks occur in Canada and as recently as December, 1955, the isolation of the virus from *D. andersoni* was reported (Brown, 1955) for the first time in this country. The ticks concerned were collected over a wide area in Alberta during 1953 and 1954. It would seem highly probable that at least a few cases in humans may occur from time to time. Periodic examination of ticks to detect the presence of the virus would be warranted.

## ARTHROPOD-BORNE ENCEPHALITIDES

Of the arthropod-borne encephalitides known to occur in North America only WEE has been recognized in Canada. In spite of the fact that WEE was first identified in horses in

<sup>1</sup> Cocksackie group viruses, with which arthropods have not been incriminated in Canada, are excluded from the present discussion.

<sup>2</sup> Throughout this paper no attempt has been made to make the list of references all inclusive.

Saskatchewan in 1935 (Fulton, 1938) and the first isolation from a human was made in 1938 (Fulton, 1941) our knowledge of the arthropod-borne encephalitides in Canada has remained relatively fragmentary. A summary of the history of WEE in Canada will form the basis for a discussion of concern over possible future developments.

Encephalitis as a disease of horses apparently has occurred in North America since at least 1847 (Cameron, 1942). In the United States the first major work on WEE began in the early 1930's following an outbreak of the disease in California (Meyer, 1932). In 1935 and 1937 outbreaks of the equine disease occurred in Saskatchewan and were followed by a 1938 epidemic which resulted in the loss of 15,000 horses (Fulton, 1941). Although the disease had not been recognized as such in Canada prior to the isolation of the virus by Fulton in 1935, it had been common for a number of years, variously described as cerebro-spinal meningitis, forage poisoning, blind staggers, corn-stalk disease and botulism. The 1935 and 1937 Saskatchewan outbreaks paralleled epidemics in Manitoba and in several of the northern states. Fulton (1941) suggests that the disease spread along a front or line of advancement from a central area.

During these horse epidemics in Western Canada the number of recognized human cases increased significantly. In 1938 human cases reached epidemic proportions in the Regina-Weyburn area of Saskatchewan. Twenty-nine cases resulted in four deaths, seven incomplete recoveries and eighteen apparently complete recoveries (Gareau, 1941). In the same year twenty-seven human cases, with six deaths resulting, were diagnosed in Manitoba. By 1941 the disease was common in the province of Alberta as well and in that year 1,094 human cases and 130 deaths were reported from the three Prairie Provinces. (McGugan, 1942; Donovan and Bowman, 1942).

These figures cannot be taken as absolute. As in the United States, deficiencies in diagnosis have confused the true picture. Donovan and Bowman (1942) refer to a number of cases originally diagnosed as polio and later determined to be encephalitis; other cases reported as encephalitis were later diagnosed as polio. In Saskatchewan during 1947-1950, 111 cases of WEE were reported; 40 percent were confirmed; 10 per cent were suspected WEE and 50 percent gave negative tests (Fulton and Burton, 1952).

Since 1941 the number of cases of WEE in humans has in general declined, but even in recent years sporadic cases have been reported from the prairie provinces and the disease may be becoming more common currently. Concerning sub-clinical cases of WEE we have relatively little information. Mitchell and Pullin (1943) examined sera from 1,013 persons in Manitoba after the 1939 and 1941 outbreaks. These sera were considered to represent a cross-section of the population and none of the patients had given any clinical evidence of WEE infection. 192 sera (19%) exhibited WEE neutralizing antibodies. In Saskatchewan on the other hand Fulton and Burton (1952) obtained only one suspicious reaction in the examination of 1,700 sera. Mitchell and Pullin (1943) also examined 101 sera from Ontario. Two gave positive neutralization tests; the one case that could be traced was a person who had been a resident of "New Ontario" where encephalomyelitis in horses was known to have occurred. Fifty sera from residents of Quebec all gave negative results. Obviously no conclusions can be drawn from these limited surveys. In view of the proven incidence of sub-clinical cases elsewhere and in view of the immunologic relations among viruses of the encephalitis group (Price, 1956; Burnet, 1955) extensive serological surveys might provide much information of value.

During the early outbreaks in Saskatchewan human cases apparently occurred only where the horse disease was present. A changing picture is reported by Fulton (correspondence, 1955). "In spite of the fact that comparatively few cases have been recognized in horses during the past seven years it would appear that the disease is becoming more common in humans. When we first determined that man was susceptible to the equine virus, we found that human cases always developed in areas where the horse disease had been prevalent and that such cases occurred after the equine outbreak had subsided. Later we noted that the human and equine disease often occurred simultaneously, but not until recently did we find human cases appearing in districts which were free from the equine infection. During the past summer very few horses developed WEE but in spite of this fact we found twenty-one cases by neutralization tests and all of these patients came from areas where the horse disease was totally absent." In this same communication Fulton made a statement which is particularly worthy of note: "... during the past few years equine



encephalomyelitis has not presented a problem of great economic importance but as you are aware a serious outbreak may occur any season."

The classic work of Kelser (1933) in proving laboratory transmission of WEE by *Aedes aegypti* (Linn.) is well-known. The later studies by Hammond and Reeves, Madsen and Knowlton, Merrill and Ten Broeck and others incriminating various mosquitoes and other arthropods as real or potential vectors of the encephalitides have been reviewed by Ferguson (1954). At the time of the early outbreaks of WEE in the Canadian West the role of mosquitoes as vectors had not been fully recognized. The first outbreaks in Saskatchewan showed no evident correlation with mosquito activity and several insects such as the stable fly, *Stomoxys calcitrans* (Linn.), were suggested as possible vectors (Fulton, 1938; McGugan, 1942).

Relatively little work has been done in Canada on vectors and reservoirs of WEE. For the most part we must rely on studies conducted in the United States. Exceptions are found in the work of Norris (1946) and that of Rempel and his associates (1946). During 1942, 1943 and 1944, Norris made attempts to recover WEE virus from mosquitoes collected in Manitoba, using the pool technique. The only positive result was from a pool of *Culex restuans* (Theo.) in 1944. No recovery was made from other pools of this species or from pools of *Aedes vexans* (Meig.), *A. campestris* (D. and K.), *A. dorsalis* (Meig.), *A. flavescens* (Mull.) *A. cinereus* (Meig.), *Anopheles maculipennis* (Meig.) (= *occidentalis* D. and K.), *Culex tarsalis* (Coq.) and *Culiseta inornata* (Will.). Norris' results were not conclusive since many of the pools were composed of small numbers of individuals and many of the mosquitoes were collected in areas where WEE was not evident or during a season in which very few cases were reported.

Indirect evidence on mosquito vectors was furnished by Rempel *et al.* (1946) in conjunction with a study of the multiple feeding habits of Saskatchewan mosquitoes. These authors concluded that on the prairies *Aedes* species could not be regarded as important vectors of WEE. They showed that the disease did not occur unless *C. inornata* (Will.) or *C. tarsalis* (Coq.) was present. These conclusions are supported by the results of studies in the United States. In connection with these Canadian studies it is of interest to note that *C. restuans* (Theo.) is not known to feed on man; *C. inornata* (Will.) feeds on domestic animals, but rarely on man, and *C. tarsalis* (Coq.) seems to prefer birds, feeding to a lesser extent on domestic animals and man (Horsfall, 1955).

In attempts to isolate WEE virus from warm-blooded hosts Canadian work again has been limited. The studies of Gwatkin and Moore (1940), Gwatkin (1941), and Gwatkin and Moynihan (1942) were not extensive. The possible recovery of WEE virus from the brain of ground squirrels was reported. Negative results were obtained in a search for the virus in a number of other small mammals, birds and a few insects.

### THE UNKNOWN AND THE FUTURE

Since the decline of WEE outbreaks in Canada during the 1940's interest in the epidemiology of the disease has been relatively dormant. However, with the mounting knowledge of WEE and other viruses of the group in the United States and elsewhere, it is becoming more apparent that in Canada we should not be completely complacent. From recent reviews by Beadle (1952), Ecklund (1953), Meyer (1953), Ferguson (1954) and Price (1956) it is obvious that our knowledge of a group of diseases barely known two decades ago is far from complete.

The remainder of this paper will deal with the direct and indirect evidence which suggests that in the future epidemics of WEE or other arthropod-borne encephalitides may well occur in Canada. It may be thought that in view of past history the word epidemic is being used in a loose sense. However, with our ever increasing standard of living, the occurrence of even a relatively small number of cases of a disease has come to be considered of significance. The importance of encephalitis quite apart from numbers involved has been cited earlier. The seriousness of the WEE problem in Canada has been reported by Fulton and Burton (1953) who showed that in some cases young children fail to develop mentally after having WEE. In addition some adults apparently recover from the disease but later become mentally ill. Fulton and Burton found WEE neutralizing antibodies in sera from a number of patients in mental institutions in Saskatchewan. They suggest that the after effects of WEE infections may be even more serious than those following poliomyelitis.



### KNOWN AND POSSIBLE VECTORS

In the western United States *Culex tarsalis* (Coq.) is recognized as the principal vector of WEE. It has been shown in this paper that whereas *C. restuans* (Theo.) is the only mosquito from which WEE has been recovered, epidemics have been associated with the presence of *C. tarsalis* (Coq.) and *Culiseta inornata* (Will.). *C. tarsalis* (Coq.) is becoming a more abundant species in the Canadian West as the result of increased irrigation developments. Breaks and seepages in such systems create breeding conditions for this species. The existence of known foci of WEE in bordering areas of the United States suggest that outbreaks of the disease are highly probable. During the summer of 1955 nearly all pools of *C. tarsalis* (Coq.) collected in the Milk River Valley of Montana, 50 miles south of the Canadian border were positive for WEE.<sup>3</sup>

There are a number of species of arthropods, native to Canada, from which one or more of the encephalitides have been isolated in nature, or which have been shown in laboratory experiments to be potential vectors. Included, in addition to species already mentioned, are: *Anopheles freeborni* (Aitken), *Mansonia perturbans* (Walk.), *Aedes dorsalis* (Meig.), *A. atropalpus* (Coq.), *A. nigromaculis* (Ludl.), *A. vexans* (Meig.), *A. campestris* (D. and K.), *Culex pipiens* Linn., *Dermacentor andersoni* Stiles, *D. variabilis* (Say) and *Dermanyssus gallinae* (DeG.).

Perhaps the biggest gap in our knowledge concerns the vector-ability of our so-called northern mosquitoes, many of which feed on man and some of which occur in astronomical numbers over extensive areas (West, 1951; Hocking, 1952; Twinn, 1952). In generally lesser numbers the range of many of these northern species extends well southward into the more populated areas of Canada and the United States.

*Aedes nigromaculis* Ludl. was shown to be a potential vector of WEE by Madsen et Knowlton (1935) and of SLE by Hammon and Reeves (1943). According to Hocking (1952) this species was found at Churchill, Manitoba, by McClure in 1936. It has not been recorded during more recent studies. There is relatively little information on the vector-ability of such a common Canadian mosquito as *Aedes vexans* (Meig.). Although no *Aedes* species has been found naturally infected with EEE, and although, as Beadle (1952) points out, the ability of a species to transmit encephalitides in the laboratory and in the field may not be the same, the possible role of *Aedes* species remains an unknown rather than being denied.

### WARM-BLOODED HOSTS

Since the complete epidemiology of the encephalitides is not known even elsewhere than in Canada, our information as to possible hosts is limited. It has been mentioned already that in Canada very few attempts have been made to isolate WEE or to detect neutralizing antibodies in warm-blooded hosts other than man and the horse. However, many of these hosts from which isolations have been made or in which antibodies have been found elsewhere (Ferguson, 1954) occur in Canada. The growing incrimination of birds in the epidemiology of the encephalitides (Kissling et al., 1951; Sooter et al., 1951; Reeves et al., 1952) is of particular interest.

### ENVIRONMENTAL CONDITIONS

The necessity of suitable environmental conditions for arthropod transmission of viruses or other disease agents is of paramount importance. Granted that much of northern Canada is probably climatically unsuitable for the extrinsic incubation of viruses in mosquitoes or other potential vectors, we are still ill-informed as to how far north WEE, for example, may occur. United States authorities have expressed interest in securing for serological examination sera from residents of northern Canada.

There exists a considerable body of information, from sources too numerous to cite here, which suggests changing faunal ranges. Some northern forms of animal and bird life have been retreating northward from former southern limits of their ranges (e.g. woodland caribou, lynx and spruce grouse). Other species, more southerly in distribution, are extending their ranges northward (e.g. bobcat, white-tailed deer, coyote, badger and moose). Insect records support this apparent trend. Chiggers have been found recently on Pelee

<sup>3</sup> Unpublished information on studies conducted at the Rocky Mountain Laboratory, U.S.P.H.S., furnished through the courtesy of Dr. A. W. A. Brown, University of Western Ontario. I am indebted to Dr. Brown, Dr. B. Hocking, University of Alberta and Dr. J. G. Rempel, University of Saskatchewan, for some of the information used in this paper.

Island in southern Ontario. The tropical rat mite, *Bdellonyssus bacoti* (Hirst), was probably recorded for the first time in Canada in 1955. The brown dog tick, *Rhipicephalus sanguineus* (Latreille), seems to have been increasing in abundance in Eastern Canada during recent years. The mite vector of Rickettsialpox, *Allodermanyssus sanguineus* (Hirst), was reported from Montreal within the past few years. Agricultural and forest insect pests records likewise give evidence of a northward movement. The debatable role of a climatic cycle in influencing these movements need not concern us here. It is, however, important that we are cognizant of these changes and may therefore expect possible increases in potential virus vectors or the introduction of vectors hitherto not recognized in Canada.

Previous outbreaks of WEE usually have been associated with hot summers. We may expect the recurrence of hot seasons at irregular intervals. Across Canada from east to west in the more populated southern portions it is unlikely that summer climate would be a factor limiting the occurrence of arthropod-borne viruses over a number of years. Until we have a more complete knowledge of the reservoirs of viruses during inter-epidemic periods, we cannot disregard the probability of future epidemics.

#### UNRECOGNIZED VIRUSES AND FUTURE INTRODUCTIONS

In serological studies conducted in conjunction with the 1941 outbreak in Manitoba the serum of one patient gave a positive neutralization test for St. Louis encephalitis virus (SLE) (Donovan and Bowman, 1942). This one serum came from a person living in a WEE outbreak area. There have been several questionable positives for SLE. In view of the widespread occurrence of SLE in the United States its apparent absence in Canada has been considered odd. The existence of at least sub-clinical cases without detection appears possible.

Similarly, although EEE has not been known to occur in Canada and in latter years apparently has had its greatest incidence in the south, it should be viewed with some concern because of the reported high mortality in humans. The probable distribution of Japanese B encephalitis (JBE) by birds on the Asiatic mainland well illustrates how wide-ranged one of these diseases may become. In this connection some concern has been expressed that JBE might be introduced into the United States (Reeves and Hammon, 1946). At least five Canadian species of mosquitoes are potential vectors. One positive neutralization test for JBE has been reported. The serum gave a negative test for WEE (Donovan and Bowman, 1942).

More and more frequently in recent years reports of unknown virus diseases and of encephalitis-like illnesses are appearing. A number of isolations of unidentified viruses have been made in Canada. While it is unlikely that many of these "diseases" would be arthropod-borne the possible occurrence of a new arthropod-disease agent association is not beyond the realm of possibility.

#### CONCLUSION

The foregoing discussion is not intended to be alarmist in tone. It is not suggested that in Canada we are on the verge of any major epidemic. Rather it has been shown that there exist various factors of the epidemiological requirements of such arthropod-borne virus diseases as western equine encephalomyelitis. Given the proper combination of circumstances we may expect at least minor epidemics.

To attempt to fill all the gaps in our knowledge would be a task beyond possibility of fulfillment. On the other hand we should not remain completely inactive and entirely dependent on the results of studies conducted elsewhere. The amount of effort that can be put forth on a study of the potential of arthropod-borne viruses in Canada must be the result of weighing the importance of the problem in terms of other public health problems and of available resources.

The view of public health officials in Canada is or should be the same as that of officials in the United States. Price (1956) has stated this view as follows: "Although the task of curbing epidemics rarely confronts us in the United States, a major responsibility of public health today consists of anticipating and preventing epidemics. This phase of preventive medicine needs to be supported by studies of the interepidemic history of infectious organisms."

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# Experimental Transmission of Yellow Fever Virus by Oriental Mosquitoes<sup>1</sup>

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## ABSTRACT

The capabilities of far eastern strains of *Aedes aegypti* to transmit yellow fever are further confirmed. Colonies from Australia, Malaya, and Taiwan (Formosa) appeared equally capable of acting as vectors. The preponderance of evidence now supports the belief that there is no mosquito barrier to introduction of yellow fever into the Far East. *A. pseudoscutellaris* and *A. polynesiensis* belong to the important *scutellaris* subgroup of the subgenus *Stegomyia* and are shown for the first time to be able to transmit the virus experimentally, though they are not potentially dangerous because of their insular distribution in the South Pacific. A strain of yellow fever virus from Trinidad proved to be more satisfactory in present studies than the Asibi or Costa Rican strains, the last possibly affected in its mosquito adaptability by 15 prior serial passages in mice subsequent to isolation. While baby mice proved unsatisfactory for donor purposes, they were found to be readily, fatally infected after mosquito bites and thus they provide an acceptable supplement to expensive monkeys in recipient tests.

## INTRODUCTION

Some three decades ago it was envisioned that yellow fever in the western hemisphere had not only been reduced to a minor health problem but that actual eradication was a distinct feasibility (Taylor in Strode, 1951). This was based on the "key center" philosophy of focal reduction in urban centers of human population in the endemic areas. Natural disappearance in rural areas was predicted on control of domestic *Aedes aegypti* and elimination of the disease in urban centers. The discovery of jungle yellow fever, different vector species in the forest canopy, as well as at ground level, and cycles in monkey hosts put a different light on this hope of eradication emphasized by recent flare-ups among people and monkeys in Central America as far north as Honduras and in Trinidad. Elton (cited by Trapido and Galindo, 1956) even "projected schedules for its [eventual] movement as far as the State of Vera Cruz in Mexico." Yellow fever is therefore still a potent problem in the tropics and subtropics of the Americas as well as of Africa.

In these days of increasingly rapid air transportation, the absence of yellow fever from southern Asia and the Far East has not only been a puzzle but of urgent concern to prevent introduction of the virus because of the potentialities of suitable mosquito vectors and of susceptible human and monkey populations existing under very exposed situations. Since monkeys have been shown to be capable of infecting mosquitoes at least 24 hours before appearance of symptoms (Hudson and Philip, 1930, and confirmed below), it appears likely that an infected air passenger might carry the virus into remote areas on a plane trip before being hospitalized. It may be a question of how long stringent vaccine regulations for travellers to the Asiatic region will be effective in blocking entrance of the virus which, of course, would not preclude introduction of an infected mosquito.

An extensive serum survey for neutralizing antibodies in residents of Australia, Java, the Philippines, Malaya, China, and India indicated no past experience with the disease, except for two unexplained positives in India (Sawyer, Bauer, and Whitman, 1937). One postulation for this absence has been stated by Whitman (in Strode, 1951) "... the failure of yellow fever to extend to India and the Orient might possibly be due to a barrier composed of *A. aegypti* with a reduced susceptibility to the virus."

A few tests of Oriental mosquitoes have shown their capabilities to transmit the virus during feeding. Dinger *et al.* (1929) showed that Javanese *A. aegypti* and *A. albopictus* are effective vectors between monkeys, both of which are important species in the Far East. Hindle (1929) also transmitted infection with an Indian colony of *A. aegypti* though suggesting these mosquitoes were not as efficient as those from Africa and Brazil. Philip (1929) showed that *A. vittatus* in Africa was an efficient vector, a species which Kumm

<sup>1</sup> From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases. This work was supported by a contract with the Chemical Corps, Fort Detrick, Frederick, Maryland.



(1931) has reviewed as occurring widely in India and as far east as Indochina. Gillett and Ross (1955) have recently employed a Malayan strain of *A. aegypti* (from the same source as used in our experiments) to transmit East African virus to a monkey.

The present report presents further evidence of the ability of certain Oriental mosquitoes to act as vectors, preliminary account of which was presented at the Tenth Meeting of the International Northwest Conference on Diseases in Nature Communicable to Man (Proc., 30 Aug. 1955, pp. 51–56).

## MATERIALS AND METHODS

### VIRUS

Three sources of virus have been utilized for the experimental studies described below.

The initial tests in 1953 employed a strain isolated by Eklund (1953) from a human case in Costa Rica in 1951. The virus had been through 15 passages in white mice and proved to be poorly adapted to mosquito-monkey transmission.

In 1954, a sample of the well-known Asibi virus was obtained through Drs. Max Theiler and Loring Whitman at the Rockefeller Institute in New York City. This virus had been through an unstated number of animal passages since its isolation in West Africa in 1928 but presumably had not been passaged in white mice. Though transmission to monkeys and mice was accomplished with this strain, inconsistent results prompted another change of strain.

In February 1955, strains from three different sources during the recent Trinidad outbreak were forwarded by Dr. Wilbur Downs of the Rockefeller Regional Virus Laboratory, Port-of-Spain. One of these, from serum from a fatal human case and virulent for monkeys, was established at the Rocky Mountain Laboratory, an aliquot portion of which had been proved before shipment from Trinidad to carry yellow fever virus.

### MOSQUITOES

Eggs of *Aedes aegypti* were received by airmail from the following persons in countries of the Far East:

Mr. J. A. Reid, at the British Medical Research Institute, Kuala Lumpur, Malaya, who has reported (1954) on a survey of that species in Malaya. Eggs were received in May 1953.

Dr. I. M. Mackerras, in July 1954, from the Queensland Institute for Medical Research, Brisbane, Australia.

Dr. D. J. Pletsch of the World Health Organization Sanitary Team, who arranged with Dr. K. C. Liang in Taiwan (Formosa) to obtain eggs of a local strain in September 1954.

Each colony was reared in a different room isolated by screen locks in the insectary and cages of uninfected lots were transferred to the experimental lock for infection and storage.<sup>2</sup> Papers with stock eggs were labelled and also stored in respective, separate rooms to prevent crossing accidents. Only Malayan *A. aegypti* were used in early transmission tests with the Costa Rican and Asibi strains of virus. The letters M, A, and F after experimental lot numbers refer to colonies from the above countries, respectively. Unless otherwise stated, mosquitoes were mostly stored during incubation after an infectious feeding at 78 to 80°C, and about 75 to 80 percent relative humidity as instrumentally controlled in our insectary (RML). Periodic blood meals on anesthetized, resistant, adult mice were provided mosquito lots between tests.

Dr. L. E. Rozeboom of Johns Hopkins School of Hygiene, Baltimore, provided colonies of adult *Aedes polynesiensis* Marks and *Aedes pseudoscutellaris* (Theo.). The former occurs in certain South Pacific islands from the Marquesas to Fiji, while the latter is known only from Fiji (Dr. Elizabeth Marks, correspondence). These represent the eastern members of the *scutellaris* group as discussed by Marks (1954), and are hereinafter referred to as the "pseudoscutellaris complex" (p).

### ANIMALS

After baby mice were shown to be unsuitable for infection of mosquitoes in early experiments, *Macaca mulatta* monkeys (referred to as "rhesus monkeys" below) were employed as donors, infected either by injection of plasma-virus, or by bites of infected mosquitoes. Baby mice have the additional disadvantage that only an inadequate number

<sup>2</sup> No rearing of western strains was in progress during these experiments.



of mosquitoes could be infected at best with tedious manipulation. The same difficulty beset attempted, direct plasma feedings through membranes and on pledgets because of lability of the virus.

Monkeys and litters of 4-day-old suckling Swiss mice were used as recipients in some of the transmission tests; Bates and Roca-Garcia (1946) had previously used baby mice for this purpose. Young (approximately 20-day-old) adult Swiss mice were used in titration studies, and for injection of mosquito suspensions or of plasma-virus. Blood was drawn from the femoral blood vessel of experimental monkeys with heparin in most instances and the plasma used after removal of the cells by centrifugation. The intensity of viremia in donors was frequently checked by titration of such plasma. Mosquitoes for injection were stunned with tobacco smoke, ground, and suspended in small amounts of diluent of 0.75 percent bovine albumin in buffered saline, pH 7.2. This diluent was also used in titrations. Dosages were 0.03 ml intracranially supplemented on occasion by 0.5 to 1 ml intraperitoneally. Suspensions were centrifuged for 10 minutes at about 3000 r.p.m. but were not treated with antibiotics. The middle layer was used for the intracranial inoculum.

Monkeys were immobilized on a padded board and the clipped, upper body exposed in cages for mosquito feeding. Some baby mice were anesthetized with diluted Nembutin for exposure, but better survival resulted when they were merely restrained by a wrapping of plastic or cloth gauze during exposure.

Cause of death in monkeys as due to yellow fever was confirmed by histopathological studies of Dr. W. J. Hadlow. All deaths in monkeys reported below were thus verified. Deaths in mice after suitable periods were assumed to be due to yellow fever and a few were checked histopathologically also or by intracranial transfer to weanling mice.

## EXPERIMENTAL OBSERVATIONS

### INITIAL TESTS WITH COSTA RICAN STRAIN

1. Baby mice donors. Two litters of 4-day-old suckling mice were injected with  $10^{-4}$  and  $10^{-5}$  dilutions intraperitoneally. Exposure to mosquito feeding, Malayan strain, was accomplished by immobilization of mice in gauze after 24 and 48 hours respectively, the latter after symptoms of incoordination had developed. All mice were dead by 60 to 78 hours.

Test feedings on individuals in separate litters were accomplished at intervals of 5, 10, and 20 days without producing any symptoms in the mosquito-bitten mice.

The tests were repeated with infectious  $10^{-5}$  dilution followed by a donor feeding after 24 hours. No test mice reacted when bitten after the same intervals above.

Unfortunately, tests by injection of mosquitoes were omitted.

2. Monkey donors. Two rhesus monkeys were each injected with 2 ml of thawed  $10^{-2}$  dilution mouse brain suspension. Neither developed a significant febrile response. Mosquitoes fed "blind" after 4 and 8 days were not infectious when suspensions were injected into 20-day-old mice intracranially. This strain was therefore abandoned for further mosquito transmission studies.

### ASIBI STRAIN

After one successful passage in 20-day-old mice with fatal outcome in dilutions up to  $10^{-5.6}$ , 5 percent mouse brain of the second passage was used to inject intraperitoneally into 2 rhesus monkeys. One showed no reaction in 3 weeks and was not used, the other had maximum temperatures of  $39.4^{\circ}\text{C}$  during 8 days, and then showed 3 days of fever followed by recovery. One lot of *A. aegypti* was fed on the 6th day and two additional lots during fever on the 9th and 10th days. After 12-15 and 17-20 days' incubation, mosquitoes from each lot were fed on a proved susceptible monkey without causing infection.

A third donor monkey was injected without mouse passage from an original ampule. It developed no rise in temperature over a period of 19 days. On the third day, blood was drawn and a "blind" feeding of Malayan *A. aegypti* on the monkey was accomplished. The plasma on this day killed all mice in  $10^{-3}$  dilution and 2 of 6 mice at  $10^{-4}$ . Plasma from blood of the 7th day in the monkey was noninfectious for mice in any dilution. Two fresh monkeys were exposed to the bites of 8 and 21 mosquitoes after 10 and 16 days' incubation in a thermal cabinet at  $85$  to  $90^{\circ}\text{C}$ . Both died of yellow fever on the 6th day after exposure; one remained afebrile, the other had one low fever of  $40^{\circ}\text{C}$  on the 4th day after exposure.

A third recipient animal (No. 9) was bitten by 9 mosquitoes of this lot, which developed fever on the 5th and 6th days but mosquitoes fed on the 6th day were later proved to be noninfectious by feeding on an additional monkey and injection into mice. However, blood drawn on the 3rd day in No. 9 killed all mice through  $10^{-3}$  dilution, the highest tested. Suspensions of mosquitoes of the original infecting lot for No. 9 also caused fatal infection in 8 and 9 days in 5 of 6 mice by intracranial injection.

Though transmission was accomplished through the bites of Malayan *A. aegypti*, further tests were unsatisfactory or showed sporadic positive results which prompted another change of strains.

#### TRINIDAD STRAIN

Results using rhesus donors infected with this strain by both plasma and mosquito bites have been much more consistent than with the prior Asibi tests; early febrile response in most instances provided a better guide for further mosquito feeding.

1. *Aedes aegypti*, Malayan strain. The original donor monkey injected with unpassaged human serum from a fatal Trinidad case, developed fever on the 4th day and was sacrificed for virus harvest. Two lots of mosquitoes were fed on the 3rd (lot 17M) and 4th (lot 18M) days. At ambient temperatures of 78–80° F, these and some subsequent lots failed to transmit the virus when tested apparently too early at 14 to 15 days. A monkey (No. 16) was infected by bites from both lots after 22–23 days' incubation however; after 3 days of fever beginning on the 3rd day after exposure, the animal died on the 6th day with gross and microscopic lesions characteristic of yellow fever. Blood of the 2nd and 3rd days of fever was infectious for new lots of *A. aegypti* and lines of infection for experimentation were continued from these mosquito lots.

Two baby mice were exposed to the bites of 4 to 6 mosquitoes of lot 18M. Both died on the 10th day while an unexposed litter mate remained well. Three others died under anesthesia during exposure.

In another test, a donor monkey (No. 19), which died on the 5th day following infected mosquito bites, developed one day of fever on the 3rd when lot 21M *A. aegypti* was given an infected feeding. Twenty-nine days later, 3 baby mice were fatally infected by bites of 4 to 6 of this lot, and after 45 days, 41 mosquitoes fatally infected a fresh monkey.

In comparable manner, after varying periods beyond an adequate incubation period in mosquitoes, and after bites of different lots and numbers of Malayan *A. aegypti*, 7 other test monkeys and 3 other groups of baby mice have been fatally infected. On occasion, shortage of monkeys necessitated biting tests in baby mice only. A summary of these and other tests is presented in Table I. Sample temperature records and virus titers for three mosquito-infected monkeys is given in Table II.

TABLE I. Successful Yellow Fever Transmissions by Oriental *Aedes* spp.

Species	Source	No. lots	No. monks.	Clinical data in days			Positive tests in mice*
				Incub.	Fever	Death	
<i>A. aegypti</i>	Malaya	13	9	3 to 6 (2 add. afeb.)	2 to 4	6 to 11 6, 7	4 lots yel. fev. to baby mice; 5 L. pos. injec.
<i>A. aegypti</i>	Formosa	2	3	3 (2 add. afeb.)	1	5 4½ ea.	Both lots pos. on baby mice.
<i>A. aegypti</i>	Australia	1	1	4	2	7	Pos. baby mice, and by injec.
<i>A. pseudo-scutellaris</i> complex	South Pacific Islands	4	2	3, 5	2, 4	4½, 10	2 lots yel. fev. to baby mice; 3 by injec.

\*Suckling mice used for biting tests with unexposed litter mates as controls. Weanling (20-day old) mice used for intracranial injection of mosquito suspensions.

Not infrequent irregularity in the clinical reaction of infected monkeys has complicated management of mosquito feeding on donors. Several monkeys have died within the usual

period of 5 to 8 days, with either no rise or a very mild rise (between 39.2 and 39.6° C) in temperature. Strong icteric tinge in the plasma at such times assists in decisions of immediate mosquito exposure. It has also been found useful to shorten the extrinsic incubation periods by temporary storage of newly-fed lots in a control cabinet for a week at higher temperatures.

Serial titrations of plasma of infected monkeys have confirmed that initial viremia not infrequently precedes fever, and also that LD<sub>50</sub> titers for mice of over 10<sup>-3</sup> are needed in donors for consistent mosquito transmission. This threshold may or may not coincide with the first day of fever, and continued rise in titer occurs thereafter to 10<sup>-6</sup> or 10<sup>-7</sup> at the height of the viremia. As indicated in Table II, at least some mosquitoes of lots 26M and 27p acquired infection from donor monkey 25, when plasma showed an LD<sub>50</sub> of only 10<sup>-3</sup> at least 24 hours before even a mild rise in temperature, thus repeating an earlier experience of Hudson and Philip (1930).

TABLE II. Sample Protocols of Three Monkeys Infected by Bites of Malayan *A.aegypti*.

Monk. No. 25 Bitten by 5 mosq. Lot 25 M			Monk. No. 32 Bitten by 116 mosq. Lots 35-36M			Monk. No. 36 Bitten by 3 mosq. Lots 37-38M		
days	temps. °C	LD <sub>50</sub> in 20-day mice	days	temps. °C	LD <sub>50</sub> in 20-day mice	days	temps. °C	LD <sub>50</sub> in 20-day mice
1	37.8		1	38.4		1	38.8	
2	38.0		2	38.8		2	38.8	
3	38.8		3	39.5		3	38.6	
4	38.4			—		4	38.6	
p.m.	38.8—bled—	10 <sup>-3</sup> *(Lots 26M, 27p infect.)		39.8—bled—	10 <sup>-4</sup>	5	38.4	
			4	39.6		6	38.8	
			p.m.	40.4—bled—	10 <sup>-6</sup>	7	37.8—bled—	10 <sup>-4</sup>
			5	40.8			moribund sacrificed necr.: yel. fev.	
5	38.5		p.m.	40.3—bled—	10 <sup>-7</sup>			
p.m.	39.3—bled—	> 10 <sup>-4</sup> *(Lots 28M, 29p infect.)	6	dead				
	—			necropsy: yel. fev.				
6	39.8							
p.m.	40.0							
7	38.7—bled—	10 <sup>-5</sup>						
p.m.	dead							
	necropsy: yel. fev.							

39.3—39.5 = prob. fever; 39.8 + = fever

\*Infection of mosquitoes proven by bites on monkeys and/or baby mice.

2. *Aedes aegypti*, Formosan strain. Using similar techniques with monkey donors, the bites of 2 lots of mosquitoes fatally infected 3 new rhesus, plus 3 groups of baby mice, unbitten control litter mates of each of which remained healthy. All 3 monkeys died on the 5th day; 2 remained afebrile, and the third had fever on one day only, during which other mosquitoes became infected as proved by subsequent successful transmission.

3. *Aedes aegypti*, Australian strain. Due to a shortage of available monkeys only one lot was tested. Lot 19A was fed on the original Trinidad donor monkey on the same day as lot 18M described above. The lot failed to transmit at 14 days but after 22 days, a monkey was infected which developed fever in 4 days and death ensued on the 7th.

Later, 2 baby mice were bitten by 6 to 8 mosquitoes of lot 19A. One symptomless suckling was sacrificed on the 7th day, and brain emulsion caused infection in 6 weanling mice which died in 9 to 11 days. The other exposed baby died on the 10th day whereas

the unbitten control remained well. Three other sucklings were killed by over-anesthesia during the initial exposure.

4. *Aedes pseudoscutellaris* complex. Four lots were fed on donor rhesus on the same day as control lots of *A. aegypti*. One small lot (No. 25p) was depleted by 50 percent in 21 days and refused test feedings on baby mice. Since no monkeys were available, a suspension of the 11 survivors was injected into 6 weanling mice; 5 died on the 10th day and last one 5 days later.

Lots 27p and 29p were fed on donor monkey No. 25 on the 4th and 5th days afebrile after infection, and fever did not occur until the day before death on the 7th day. After 17 and 18 days' incubation, 4 and 7 of these mosquitoes, respectively, bit fresh monkey No. 26; this animal developed 2 days of fever on the 3rd and 4th days and died on the 5th. The fourth lot (33p) and a lot of Malayan *A. aegypti* fed on the 3rd day on No. 26 and both became infected as proved by later transmission. Four mosquitoes of Lot 33p bit monkey No. 27, and 3 more 2 days later; this animal developed fever, viremia of  $10^{-6}$  LD<sub>50</sub> and died on the 10th day after initial exposure. Necropsy findings were typical. Each of 4 surviving insects was later injected into separate weanling mice, all of which died in 6 to 9 days.

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## DISCUSSION

R. LEVI-CASTILLO. We found in Ecuador the same clinical conditions in an infection with jungle yellow fever in Saimiri monkeys directly from *Haemagogus spegazzinii falco* mosquitoes taken from nestlings of wild birds in the jungle. *H. spegazzinii falco* was found in great quantities and at ground level. Larvae and pupae of this species were found in the palm tree chontaruru (*Aiphanes caryotifolia*) living in the hollow trunk filled with rainwater, flying from there to bite man.

C. B. PHILIP. Dr. Trapido, what is present thinking regarding Elton's projected schedule of progress of yellow fever as far north as Vera Cruz?

H. TRAPIDO. Since demonstration of vectorship of *Haemagogus equinus*, *H. mesodentatus* and *Sabethes chloropterus* in Guatemala, it appears likely yellow fever virus will proceed to the Mexican Gulf coast.

W. H. R. LUMSDEN. The barrier for yellow fever between Africa and Asia appears to be on the East African Coast. Positive neutralization tests occur in Galagos on the East African Coast but in monkeys or man they are naturally very rare or absent.

A. MICHAEL DAVIES. It might be interesting to doubly infect mosquitoes with yellow fever and dengue which do show cross immunity. In this connection, does Dr. Philip think that the lack of spread of yellow fever to India and Egypt might be attributed to the presence of dengue in those countries?

C. B. PHILIP. I don't believe that protection against yellow fever by a previous attack of dengue has been proved.

# Survival of Mouse Poliomyelitis Virus in Living Invertebrate Hosts

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## ABSTRACT<sup>1</sup>

*In a search for a living invertebrate that might serve in the capacity of a culture vehicle and medium for different viruses, mouse poliomyelitis virus strain FA having a 50% endpoint of  $10^{-5}$  was injected into the hemocoels of several invertebrates, and at intervals thereafter suspensions of their ground bodies were titrated in mice to determine levels of virus potency. The titer dropped steadily from the highest of under  $10^{-5}$  to near 0 at the end of the survival period. Maximum survival times were: a cricket, *Acheta assimilis* F., 21 days; a snail, *Lymnaea stagnalis* (L.), 7 days; a snail, *Biomphalaria (Australorbis) glabratus* (Say), 14 days; three conenose bugs, *Rhodnius prolixus* Stal, 21 days; *Triatoma phyllosoma pallidipennis* (Stal), 28 days; and *Triatoma infestans* (Klug), 21 days. Certain implications as to possible use of living invertebrates as culture vehicles for viruses and as to possible roles in survival and transmission of human poliomyelitis virus were discussed.*

<sup>1</sup> For complete text see *Jour. Infect. Dis.* 100: 12-16. January-February 1957





# Vector Problems Associated with the Development and Utilization of Water Resources in the United States

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## ABSTRACT

*Water resources vector problems are considered in two groups: (1) Those associated with impounded waters, and (2) those associated with irrigated areas. Principal types of impounded waters are multipurpose reservoirs, water supply reservoirs, fish and wildlife ponds, recreational lakes, farm ponds, log ponds, and sewage lagoons. Impounded water vector problems include malaria, encephalitis, and pest mosquitoes; horseflies and deerflies; midges; and (in marginal recreation areas) flies, fleas, ticks, and mites. The public health importance of species which do not transmit disease is emphasized.*

*The most important vector problems of irrigated areas are encephalitis, malaria, and pest mosquitoes; biting gnats; and blackflies. Good irrigation practices are mutually beneficial to mosquito control and agriculture. Preventive or source reduction measures are given major emphasis in the control of the most important vectors associated with water resources developments, but supplementary use of insecticides is also necessary.*

## INTRODUCTION

Adequate development and wise utilization of water resources has become of vital importance to social and economic progress in the United States. Municipal, industrial, and agricultural demands for water have rapidly expanded during the past two decades. It is estimated that by 1975 our population will have reached 200 million and we will need at least 350 billion gallons of water per day, almost twice the amount used in 1950 (Presidential Advisory Committee, 1956). To meet this need there is underway a greatly accelerated water resources development program. Public Health is one of the various interests involved in this program, and is concerned chiefly with municipal and industrial water supply planning, pollution control, and vector control. The present paper reviews the mosquito and other vector problems associated with the development and utilization of water resources in the United States.

The development and utilization of water resources usually involves the inundation of land by impoundment or diversion of the natural water flow. The ecological changes which accompany this transformation from a terrestrial to an aquatic habitat result in the production of a variety of arthropods of public health importance. Of these, mosquitoes are undoubtedly the most important, but various other groups are also involved; these include horseflies and deerflies (Tabanidae), blackflies (Simuliidae), biting midges (Heleidae), and chironomids (Tendipedidae). Recreational use of the waterside also brings the public into close contact with other arthropods, such as ticks, mites, and fleas, and these must be taken into consideration as a part of the vector problems associated with the utilization of water resources.

In the modern concept, health means not merely freedom from infirmity but complete physical, mental, and social well-being. Accordingly, an arthropod of public health importance is defined in the present paper as any species which transmits disease or otherwise interferes with man's physical and mental comfort. For brevity's sake, "vector" will be used to mean an arthropod of public health importance, although some may object to this broadened use of the term.

The vector problems associated with permanent or semi-permanent impoundment of water and the methods for their solution are usually quite different from those associated with temporary flooding by irrigation. These two types will, therefore, be discussed separately.

## VECTOR PROBLEMS ON IMPOUNDED WATERS

Artificial water impoundments range in size from huge reservoirs with thousands of acres of water surface to small farm ponds less than an acre in extent. The various types include: (1) relatively large multipurpose reservoirs built for hydro-electric power genera-

tion, and/or flood control, navigation, and related uses; (2) municipal and industrial water supply reservoirs; (3) fish and wildlife ponds; (4) recreational lakes; (5) farm ponds; (6) log storage ponds; and (7) sewage lagoons. The factors which determine the vector problems associated with these impoundments are basically similar, though of different magnitude.

#### MALARIA CONTROL ON IMPOUNDED WATERS

One of the major vector problems related to impounded water has been the production of malaria mosquitoes. Of the various anopheline mosquitoes in the United States, only two, *Anopheles quadrimaculatus* in the East and *A. freeborni* in the West, have been of general epidemiological importance in the transmission of malaria. *A. quadrimaculatus* is the most important of the two and has been primarily responsible for the traditional endemic malaria of the southeastern States. It breeds in relatively permanent collections of fresh, clear, quiet, warm water, including the shallow margins and backwater of reservoirs, with an abundance of vegetation and/or flottage. Long before the discovery of the transmission of malaria by mosquitoes it was recognized that outbreaks of the disease were often associated with reservoirs. After the turn of the century there were a number of serious malaria epidemics in the southeast centered around impounded water projects, and these foci of transmission persisted for many years. An interesting account of this early period and the malaria prevention and control measures which were ultimately developed for impounded water have been presented in a joint publication of the United States Public Health Service and the Tennessee Valley Authority (1947).

Shortly after its creation in 1933, the Tennessee Valley Authority employed a technical staff in the fields of medicine, biology, and engineering to develop a malaria control program for the great chain of reservoirs which it was to construct on the Tennessee River and its tributaries. Surveys at that time showed an average parasitemia rate of 35 percent, and in some areas two-thirds of the population had malaria parasites in their blood (Gartrell, 1951). With the economic facilities available it seemed almost impossible that malaria would ever be brought under control. However, under the persistent and capable leadership of the late E. L. Bishop, Director of Health, malaria was essentially eradicated from the Valley, and the Tennessee Valley Authority program is now recognized as a model throughout the world. The Tennessee Valley Authority program placed major emphasis upon building mosquito prevention or source reduction methods into the projects. These consisted of a combination of measures, including reservoir preparation, shoreline maintenance, larviciding and adulticiding, mosquito proofing, permanent shoreline improvement (source reduction), and water level management (Derryberry and Gartrell 1952). The latter two measures have been the backbone of the program. As major anopheline breeding areas were progressively eliminated, more complete reliance could be placed upon water level management, resulting in greatly reduced unit costs with only supplemental use of repetitive measures.

Water level management for mosquito control on impoundments such as those in the Tennessee Valley involve the following elements: a spring flood surcharge to strand drift and flottage; a constant pool phase to limit early season invasion of marginal vegetation; a period of cyclical fluctuation to interrupt mosquito production; and finally, seasonal recession with or without cyclical fluctuation (Hess and Kiker, 1944). The combination used on a particular project is adapted to existing physical limitations, and the multipurpose functions of the system. The Tennessee Valley Authority water level management schedules for mosquito control are an excellent illustration of the effective application of biological principles within a framework of engineering and operational limitations. The effectiveness of water level recession, for example, is dependent upon the degree to which it reduces the intersection line (Hess and Hall, 1943), one of the dominant factors in the larval ecology of *A. quadrimaculatus* (Fig. 1).

Small reservoirs, as well as large ones, may create a malaria hazard. This was well illustrated by the outbreak of 35 cases of malaria among Camp Fire Girls in California in 1952 at Lake Vera, a small impoundment of only 15 acres (Brunetti *et al.*, 1954). Likewise, the same principles of prevention and control apply to small impoundments. In the case of Lake Vera, effective control was achieved by permanent elimination of the major mosquito breeding areas by deepening and filling, supplemented by the application of mosquitocides (Fontaine *et al.*, 1954).

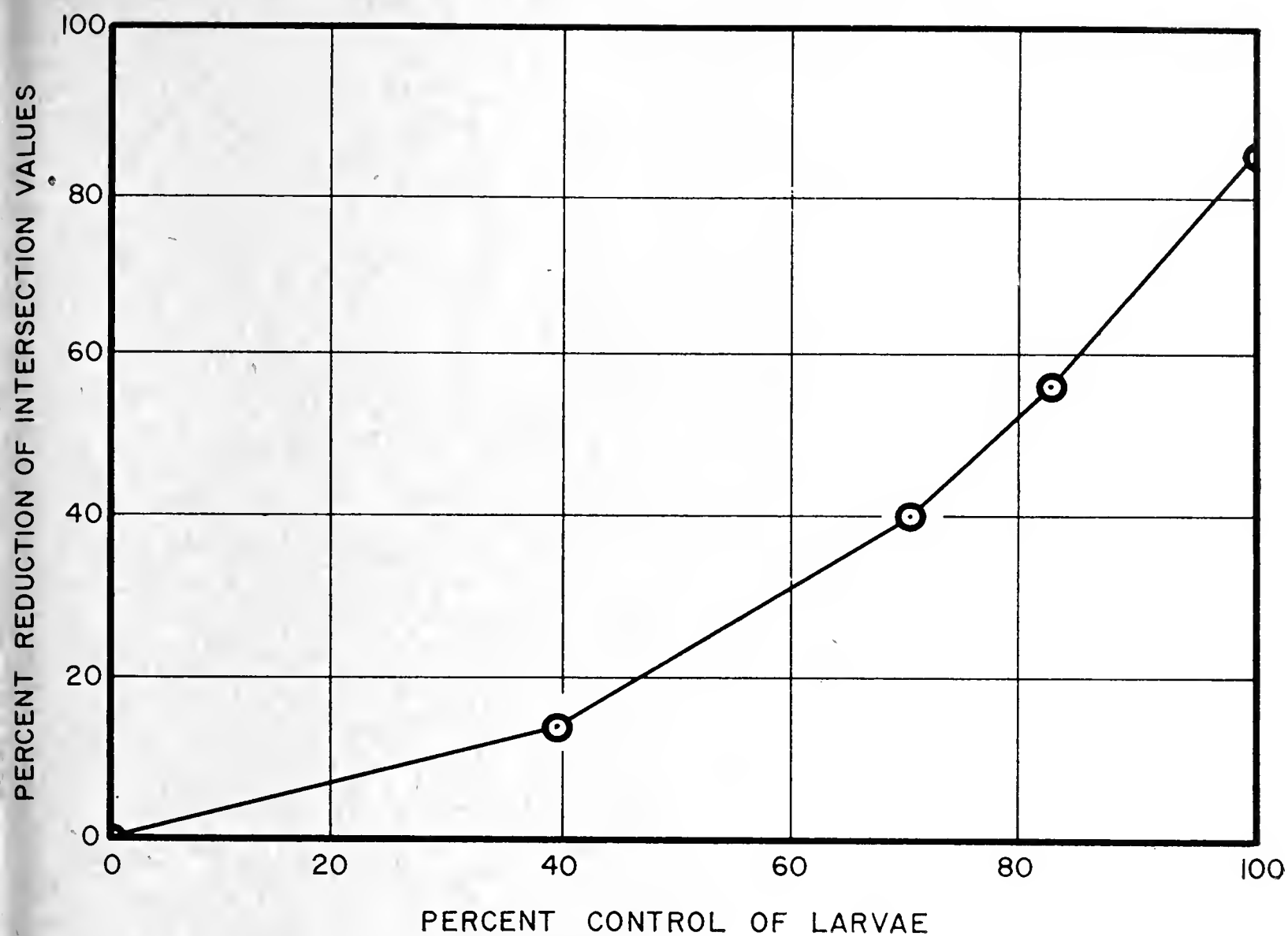


Fig. 1. Relationship between reductions in intersection values and reductions in larval densities. Graph based on 3,500 square-foot dips, Kentucky Reservoir, June 6 to August 7, 1946. (From Malaria Control, July-December, 1946, Tennessee Valley Authority).

#### OTHER IMPOUNDED WATER VECTOR PROBLEMS

Now that malaria has been largely eradicated from the United States, more attention is being given to other impounded water vector problems. Certain pest mosquitoes, such as *Culex erraticus*, occur in the same breeding areas with *A. quadrimaculatus* and may be controlled by the same measures. Other species, such as the floodwater mosquito, *Aedes vexans*, are produced under quite different circumstances. When the reservoirs are filled in the spring, the overwintering eggs along the shoreline hatch and a heavy emergency of mosquitoes may result. With progressive seasonal water level recession, no additional production will occur; but if summer rains or floods again fill the reservoir, additional broods will be produced. The extensive flight range of this species (commonly 15 to 20 miles) complicates the problem of its control and often necessitates the use of temporary space adulticiding measures.

Some recent observations by the Public Health Service in northern Montana illustrate the serious public health hazards which may be created by pest mosquitoes (Hess and Quinby, 1956). A major portion of the 133 families interviewed reported that mosquitoes seriously interfered with the healthful outdoor activities of both children and adults. Most individuals reported some type of injurious effects from mosquito bites, such as itching, swelling, scratching, and secondary infections. Over 50 percent of the individuals examined by the interviewing physician suffered abrasions from the scratching of mosquito bites, and 40 percent of the individuals living in substandard housing showed signs of secondary infection. Allergic reactions and secondary infections were frequently so severe that they required the attention of a physician or even hospitalization.

A unique type of mosquito control problem is created by log ponds in the Pacific Northwest. These ponds vary from a fraction of an acre to a hundred or more acres in extent and are usually heavily polluted with organic extracts and debris from the logs which are passed through them. They often produce excessive numbers of *C. tarsalis* (the principal encephalitis vector in the far West), *C. stigmatosoma*, *C. pipiens*, *Culiseta incidens*, and where there is marginal vegetation such as cattails, *Mansonia perturbans*. Control has

proved to be difficult, and current investigations are underway to elucidate the larval ecology and to develop practicable control measures.

The use of oxidation ponds or sewage lagoons for community waste disposal is rapidly expanding in the central and western States. If these lagoons have shallow vegetated margins they produce large numbers of mosquitoes, particularly *Culex pipiens*, *C. quinquefasciatus*, and *C. tarsalis* (Eads, 1956). The primary solution to the problem would seem to be proper construction and maintenance. They should be built with clean abrupt shorelines and subsequently kept free of vegetation and trash. The Public Health Service is presently making a survey of the mosquito production potentials of sewage lagoons with a view of incorporating vector prevention into their design and operation.

Fish and Wildlife impoundments frequently receive adverse criticism because of mosquito production. The same principles of vector production and control apply to them as to any other impoundment. Conflicts of interest usually center around limitations on water level management and restricted use of insecticides. Wildlife and vector control interests are not, however, always antagonistic and a cooperative approach to the problem may often result in the development of mutual interests (Wiebe and Hess, 1944). Where wildlife ponds produce excessive mosquitoes and preventive or corrective measures are not practicable, they should be located beyond flight range of human population centers or recreational areas.

Several species of Tabanidae (principally *Tabanus* and *Chrysops*) may be produced in sufficient numbers around the margins of impounded waters to create a vector control problem. Many are vicious biters and may sometimes be involved in the transmission of diseases, such as anthrax and tularemia. Relatively little is known about the biology and control of tabanids. Some satisfactory results have been obtained by larvicidal treatment of shoreline breeding grounds (Brown, 1951).

Public demands for outdoor recreation areas are steadily increasing, and the shores of impounded waters are favorite sites for summer cabins, campgrounds, and picnic areas. This brings the public into contact with vectors of aquatic origin as well as those of terrestrial origin, such as flies, ticks, mites and fleas. The public health importance of the terrestrial vectors involves biting and annoyance as well as a variety of human diseases including typhoid, bacillary dysentery, Rocky Mountain spotted fever, Colorado tick fever, tularemia, relapsing fever, tick paralysis, typhus and plague. It is, therefore, becoming increasingly evident that the temporary inhabitants of recreational areas around impounded waters must be protected from terrestrial vectors as well as those of aquatic origin. Preventive sanitation measures are important, particularly with regard to fly control. In the case of ticks and mites, residual applications of insecticides to the grass and shrubbery of picnic areas and along pathways is usually effective. Where plague is enzootic, the general public appeal of chipmunks, ground squirrels, and squirrels dictates that control should be aimed at the flea vectors rather than the rodent hosts. The extensive reservoirs of plague among wild rodents in the western States makes it expedient that we give increased investigative attention to sylvatic plague control. In the southern States where murine typhus is endemic, effective use has been made of insecticidal dusts to control the flea vectors (Hill *et al.*, 1951).

In planning vector control for impounded water projects it is common practice to classify the shoreline below top pool level as Type I, II, or III in accordance with the relative mosquito production potentials (U.S.P.H.S. and T.V.A., 1947). This classification is important with regard to zoning the shoreline for human use, and recreational areas should be located in zones of lowest vector potential. In establishing these recreational areas, consideration should also be given to the vector breeding potentials of adjacent areas outside of the reservoir. Thus, the final zoning of impounded water shorelines should be based upon an analysis of both the terrestrial and aquatic vector production potentials.

Among other pestiferous insects which may be produced in impounded waters are small non-biting midges (Tendipedidae) and phantom midges (Chaoborinae). They are often attracted to lights in tremendous numbers and create great physical annoyance. An example is the Clear Lake gnat, *Chaoborus astictopus*, of California which has seriously affected the resort areas of Lake County. Injection of preemulsified TDE (dichloro-diphenyl-dichloroethane) into the lake water has given effective control without injuring the fish life (Brydon, 1955).



## IRRIGATION VECTOR PROBLEMS

Some of the most rapid expansion in water resources development in recent years has been in the field of irrigation. This is particularly true in the western States where the total acreage of irrigated land has increased from 17 million acres in 1939 to almost 30 million acres in 1956. Even in the more humid East there is a marked increase in the use of irrigation water to supplement natural rainfall. In addition to storage reservoir irrigation systems, there is widespread use of deep wells to provide irrigation water in many western states. There is also an increasing tendency to use sewage effluent for irrigation where other water is scarce. The rapid expansion in irrigation developments has been accompanied by proportionate increases in the production of mosquitoes, which are now creating serious public health problems in many irrigated areas. Encephalitis is one of the most important of these public health problems.

### ENCEPHALITIS IN IRRIGATED AREAS

Encephalitis is now the most important insect-borne disease in North America. Scattered outbreaks occur over the United States during the summer months with occasional severe localized epidemics. Three distinct viruses are the principal etiologic agents of the arthropod-borne encephalitides in this country: Western equine (WEE), Eastern equine (EEE), and St. Louis encephalitis (SLE). As the names imply, WEE and EEE produce clinical symptoms in horses as well as humans, but SLE infections in horses are subclinical. EEE has also been responsible for serious epizootics among pheasants in the Northeast. WEE and SLE are confined principally to the western half of the United States, although SLE outbreaks have occurred as far east as Indiana and Kentucky; EEE occurs chiefly in the States bordering the Gulf and Atlantic coasts.

The chief summer reservoir for the encephalitis viruses appears to be wild and domestic birds, with mosquitoes transmitting the viruses from bird to bird. Less is known about the long-term or winter reservoirs. Man and horses are accidental victims and receive their infection through the bites of mosquitoes. It is generally agreed that in the far western States *Culex tarsalis* is the primary vector of WEE and SLE with other species playing secondary roles. There is increasing evidence that house mosquitoes (*Culex pipiens* and *C. quinquefasciatus*) may have a primary role in the transmission of SLE in the central States. Less is known about the vectors of EEE but *Culiseta melanura* appears to be important in the basic infection chain (bird-mosquito-bird) and salt-marsh mosquitoes may possibly be involved in transmission to humans.

*C. tarsalis*, the principal encephalitis vector in the far West, breed prolifically in seeps, standing pools, roadside ditches and other semipermanent aquatic habitats associated with irrigation projects, particularly those which have been improperly constructed or inadequately maintained. Observations by the Public Health Service at the Angostura Project in South Dakota showed that mosquito production in the area was increased almost one-hundred fold due to excessive seepage from the main distribution canals and laterals when the project was put into operation (Rainey, 1955). Three-fourths of the mosquitoes produced were *C. tarsalis*.

Other mosquitoes which breed abundantly in irrigated areas have been found naturally infected with encephalitis and may serve as secondary vectors. Among the most important of these are *Aedes vexans*, *A. dorsalis*, and *A. nigromaculis*. Like *C. tarsalis*, their production is greatly increased by faulty irrigation practices.

Good irrigation and water management practices drastically reduce or eliminate the production of encephalitis or other irrigation mosquitoes by reducing the extent and duration of the ponded waters in which they breed (Meyers, 1956). These practices involve good distribution systems, proper land preparation, conservation irrigation, and adequate drainage systems. Since the spring of 1955 the United States Public Health Service and the Agriculture Research Service have been conducting cooperative investigations in northern Montana to develop and demonstrate improved irrigation practices which will prevent mosquito production and increase crop yields. The results to date are quite encouraging (*op. cit.*). Thus, the basic philosophy on irrigation mosquito control is that preventive or source reduction measures offer the ultimate solution for the major problem, but that interim and supplementary use of insecticides is a necessary adjunct.

The development by mosquitoes of high levels of resistance to chlorinated hydrocarbon insecticides has had an important effect upon control operations, particularly in the far West and the Southeast. In most of these situations organophosphate insecticides have been successfully substituted for the chlorinated hydrocarbons. In many areas, such as the intermountain region of the West, resistance has not yet appeared and chlorinated hydrocarbons are still highly effective. In such areas, preflood and postflood residual larviciding offers considerable promise for irrigation mosquito control (Smith and Shultz, 1956).

#### OTHER IRRIGATION VECTOR PROBLEMS

Irrigation mosquitoes may include both disease vectors and pest species. The pest species may create serious public health hazards, as indicated in the section on impounded water vector problems (Hess and Quinby, 1956).

Mosquito production on irrigated fields occurs most frequently on pastures, hay meadows, and other close-growing forage crops. Relatively little breeding occurs on fields planted to row crops since these crops cannot survive the period of flooding required for mosquito larvae to complete their development.

The irrigated rice areas of California and the Mississippi Delta produce several species of mosquitoes including the principal vectors of malaria and encephalitis. Semipermanent flooding produces *Anopheles quadrimaculatus* in the Delta region and *A. freeborni* and *Culex tarsalis* in the California rice fields (Henderson, 1952). Intermittent flooding produces primarily *Psorophora confinnis* and *P. discolor* in the Delta rice fields and *Aedes* spp. in the West if the water stands for a few days. Use of granular insecticides and preflood larviciding with chlorinated hydrocarbons showed promise in early tests, but the development of resistance has largely rendered these materials ineffective against rice field mosquitoes (Mathis, et al., 1955). Recent observations in California suggest the possibility of biological control through the use of blue-green algae of the genus *Anabaena* (Gerhardt, 1955). More emphasis should be given to the development of improved cultural control measures for rice field mosquitoes.

Small biting gnats of the family Heleidae (*Culicoides*, *Leptoconops*, and *Ceratopogon*) breed in the damp areas of many irrigated valleys as well as salt marshes and other wetland areas. They are vicious biters and often produce severe reactions and vesicular lesions. Application of DDT and other chlorinated hydrocarbon larvicides to breeding areas has shown promise for the control of some species. Insecticidal treatment of window screens, repellents, and space sprays have also been used (Brown, 1951; Rees and Smith, 1952). Much more work is needed on the biology and control of this group of pests.

Blackflies (Simuliidae) plague man and livestock in many parts of the United States with their painful and irritating bites. Although they normally breed in natural streams, they may also be produced in canals and drop structures of irrigation systems (Edmunds, 1954). Application of DDT to the breeding streams is highly effective in concentrations as low as 0.1 ppm for a 15-minute period (Brown, 1951).

Several arthropod pests discussed under impounded waters may also be produced in irrigated areas. These include various mosquito species common to both types of areas (such as *C. tarsalis* and *Aedes vexans*), horseflies and deerflies, gnats and midges, and various terrestrial vectors.

The basic principles governing vector production and control on irrigation storage reservoirs are the same as for other impoundments. They are, however, less likely to create vector problems than the multipurpose reservoirs of more humid areas because the shorelines are generally more arid and sparsely vegetated, and they usually have a wide seasonal recession of water level.

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## DISCUSSION

J. FRAGA DE AZEVEDO. I ask Dr. Hess if there are present in his country some measures of control, in particular biological measures affecting the vector mosquitoes?

A. D. HESS. It is very important that we conduct fundamental research on the natural history of encephalitis, including the ecology of the hosts and vectors. The findings will give us the best approach to either biological or chemical control measures. Host or reservoir control is a possibility as well as vector control.

C. B. PHILIP. Correction: that horses were shown to develop at least mild symptoms after experimental infection, by Cox *et al.* We also found horses during Colorado equine epizootic with antibodies.

A. D. HESS. Yes, we often find SLE antibodies in horses, but St. Louis does not produce the severe clinical outbreaks and deaths among equines comparable to those produced by WEE and EEE.

R. LEVI-CASTILLO. We have in Ecuador Venezuelan equine encephalitides and we have been controlling it with Dieldrin, 1 gram active substance per square meter.

A. D. HESS. It is interesting to study the vectors and reservoirs of disease and to control the reservoirs rather than the vectors.



# Today's Tactics Against Malaria and its Vectors

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## ABSTRACT

Over the centuries, the attack on malaria has developed principally along five lines: avoiding malarious localities, doing away with aquatic habitats of the vector, destroying aquatic stages, attacking the plasmodia in man, and killing adults of vector anophelines. All of these methods remain in use but emphasis has shifted markedly in the past 10 years. Today, anti-malaria drugs are coming into increased use and, more important, malaria is being controlled over wide areas, often to the point of eradication, by residual spraying of chlorinated hydrocarbon insecticides. Nearly 300 millions of the some 930 millions who are estimated to live in malarious areas of the world, are now under more or less routine protection from the disease. The World Health Organization is giving inspired leadership and assistance to world-wide malaria eradication. Much bilateral and international aid money is being contributed to nation-wide programs of residual spraying. Malaria is still of great world importance, with probably some 200 millions of cases occurring annually. But the attack on this disease has shown great acceleration in recent years.

## MALARIA DISTRIBUTION AND PREVALENCE

Very likely the widest world distribution of malaria occurred sometime between 1880, when the disease was still endemic in southern Canada, and 1920, when it approached the Arctic Circle in Russia. Hirsch (1883) set the northern limit of malaria in the Americas at Kingston, Ontario, with occasional epidemics at Lake St. Peter, on the St. Lawrence River. Davidson (1892) stated that Kingston and Toronto on Lake Ontario were undoubtedly malarious, although in a minor and diminishing degree. Today, malaria is no longer endemic in Canada, in fact it has been almost completely eradicated north of Mexico. The vector anophelines are still common. *Anopheles quadrimaculatus* is found throughout the eastern and central United States and is in Canada as far north as the southern fringes of Quebec and the Ottawa Valley in Ontario. *Anopheles freeborni*, another efficient vector, is present in British Columbia and southwards in Oregon, Washington, California, and elsewhere, mostly west of the Continental Divide. But, in the absence of gametocytes, no malaria transmission occurs.

As to malaria prevalence, data are so poor that in making estimates one must take into account personal acquaintances and communications, as well as official reports, published and unpublished. From these sources, I (Russell, 1956) have estimated that of the world's total population of some 2.65 billions, no fewer than 1,070 millions live in areas where malaria is or recently has been endemic. According to the latest reports, some 375 millions were being routinely protected to some degree from malaria, throughout the world in 1955. But, even so from 200 to 225 million clinical cases probably occurred, with some 2 million deaths directly due to the disease. This is a huge total but it is very likely 100 million cases fewer than the annual total 10 years ago: so there has been progress.

## SOME HISTORICAL NOTES

Looking back, one notes the gradual emergence of five principal classes of malaria control measures: (1) avoiding malarious localities; (2) removing or altering waters associated with fevers; (3) destroying mosquito larvae; (4) administering medicines to curb or knock out the pathogen; (5) killing adult mosquitoes.

These measures were applied empirically until Laveran discovered the plasmodia in 1880 and Ross, Grassi, Bastianelli, and Bignami laid bare the underlying facts of mosquito transmission, in 1897-1898. Then, Stephens, Christophers, and James, in India in 1902, demonstrated clearly that only certain species of *Anopheles* transmit malaria naturally. Basing his practice on this fact, Watson in Malaya was first to control malaria by species sanitation, in 1903. During the next ten years Gorgas, in the Canal Zone, applied the new principles on a large scale, and was spectacularly successful. Thereafter the attack on malaria came into much sharper focus.



The five classes of malaria control developed somewhat as follows:

1. *Avoiding malarious localities*: Even in ancient times, the avoidance of certain localities was recommended as a practical way to prevent fevers. For example, Vitruvius, in the first century B.C., advised farmers to site their houses "away from marshes, the poisonous exhalations of which exert a morbid influence on man." People in many lands have instinctively practiced this measure of control. A good example is seen in the Philippines where for centuries Igorot, Bontoc, and other tribes of the northern mountains and Ilocano, Tagalog, and other groups of the nearby lowlands have avoided the intervening fever-ridden foothills where, as we now know, the principal malaria vector, *A. mimicus flavirostris*, has its preferred breeding places, along the edges of small streams. So thoroughly has this fever belt been avoided in the past that some anthropologists believe malaria to have been a prime factor in maintaining the sharp sociologic differentiation between the mountain folk and plainsmen of Luzon.

Today, with malaria eradication technically and financially practicable almost anywhere, malarious localities need not be avoided. They can be made safe and put to good use.

2. *Removing or altering waters associated with fevers*: By the time of Hippocrates, in the 5th and 4th centuries before Christ, an association between marshes and disease had been recognized. In early Roman times men like Varro, Columella, and Vitruvius were advocating drainage to combat "morbid influences." Since then, through the centuries, this measure has lowered the incidence of intermittent fevers in many countries, although most often indirectly, as a by-product of agricultural and urban bonification. Today, drainage for malaria control is considerably less practiced than formerly, probably to the point of neglect of a useful measure. No doubt in many areas where antimalaria drainage would be a sound investment it has been put out of mind by the dramatic successes of DDT.

3. *Destroying mosquito larvae*: That method of combatting malaria that gave greatest promise of success during the first half of this century was the use of insecticides to destroy the aquatic stages of anophelines. Oil larvicides were used increasingly up to and through the period of World War II. Paris green came into use after 1921 and it was very popular, applied by hand, by small blowers, and from airplanes. Then, during the last war, DDT became available. It greatly increased the effectiveness and ease of application of oil, and the combination was extensively employed. But today, in malaria control, larvicides have been largely supplanted by adulticidal sprays, which are usually more economical and effective. Here and there, larvicides are still logical weapons. For instance, in Jordan, *A. sergenti*, the principal malaria vector, seldom rests day or night in human habitations. It prefers caves and these can not be economically subjected to adulticidal spraying. Here residual larviciding with Dieldrin has been an effective measure of malaria control. Elsewhere, in certain urban areas, larviciding is still the most practical method of attacking malaria, frequently also of destroying the larvae of pest mosquitoes.

4. *Administering medicines*: Man has always been willing to "take something" to ward off fevers. From incredibly nasty mixtures of Babylonians, Egyptians, and Chinese to hot buttered rum in New England, and from decoctions of cinchona bark through quinine tablets to modern synthetic antimalarials, the list is long and the end is not yet.

But mass treatment is a difficult and expensive public health measure to apply, whatever the medicine. In the past, it has never been economically practicable to control malaria by drugs on any but a limited scale or in special circumstances. Today, however, drugs are very important as adjuncts to residual insecticiding in malaria eradication projects. Of particular importance is the fact that as the incidence of the disease falls to minimal levels, residual pockets can be hunted out and the plasmodia exterminated in man with the new antimalarials. These modern remedies may have great value throughout an eradication project and are essential in the final stages.

5. *Killing adult mosquitoes*: The first measure that had some preventive effect on malaria was, I suppose, the slapping of mosquitoes because of their annoying method of obtaining food. Probably smoke smudges and homemade repellents were also in early use by primitive peoples. So, too, bed nets are an ancient device to thwart mosquitoes, and they have had and continue to have wide usefulness in the tropics. Screening of windows, doors, and porches, especially in the southeastern United States, has been an important measure of malaria control, although never much used elsewhere.

After the discoveries of Ross and the Italians, it became obvious that some degree of malaria control might derive from the destruction of the vector anophelines in their adult resting places. Gorgas and Le Prince, for example, in the Canal Zone, noted definite help from the systematic killing of adult mosquitoes in laborers' quarters. Then, in 1927, the Malaria Commission of the League of Nations recommended that housewives make the killing of house-haunting mosquitoes a part of their daily cleaning task. This method gained increased attention when Park Ross and De Meillon, in South Africa, in 1935, reported successful malaria control by routine space-spraying with a pyrethrum insecticide inside houses where a majority of the vectors, *A. funestus* and *gambiae*, rested in the daytime. The measure was then tried in India and found to be a thoroughly economical and effective means of interrupting malaria transmission by *A. culicifacies*. In the United States, household pyrethrum sprays against adult mosquitoes have been extensively used and remain popular.

Then in 1939 and the next few years, came DDT (dichlorodiphenyl-trichlorethane), BHC (benzene hexachloride), and Dieldrin,—all synthetic chlorinated hydrocarbon insecticides with remarkable residual killing properties, and with ease and cheapness of application. Today, the most effective and popular measure for attacking malaria is the killing of vector anophelines inside houses by the use of residual DDT, BHC, or Dieldrin.

### MALARIA ERADICATION

Malaria eradication, by general agreement, implies the ending of transmission and of the reservoir of infective cases in a campaign limited in time and carried to such a degree of perfection that when it comes to an end there will be no resumption of transmission. To eradicate malaria is to make it nonendemic. On the basis of a definition prepared by the National Malaria Society some years ago, it is understood that malaria is no longer endemic in a given area when for three consecutive years there has been no natural transmission of the disease within that area. Of course, it is assumed that the area is clearly delimited and reasonably large and that there has been active, routine, and skilled surveillance to detect locally acquired infections. This definition properly excludes any reference to mosquito densities. Obviously, malaria eradication is not the same as vector eradication. The latter implies complete extirpation of the malaria-carrying species of *Anopheles* from a given area, a task usually not technically or financially practicable.

Observation demonstrates that when all factors are favorable, one year's spraying with residual insecticides will stop malaria transmission in an area. When complete freedom from transmission has been maintained for three consecutive years, malaria is no longer an endemic disease in that area. The reservoir of plasmodia, because it has not been replenished for three years, will be practically dry. Intelligent use of modern antimalaria drugs will end the menace of the few remaining human infections. But, of course, only seldom are all factors favorable. Experience indicates that to obtain 3 consecutive years of no transmission at least 4 years of active spraying will be required. The spraying period is followed by another 3 or 4 years of active surveillance by trained "vigilance teams" such as are now operating in Ceylon. These teams patrol the entire area, investigate all cases of fever, administer therapy to those with malaria, reapply insecticides if necessary. Surveillance constitutes the main defense in the final stages of an eradication project, when spraying has ended. After the periods of spraying and surveillance have been completed, normal health department activity can be depended on to deal with any introduced cases of malaria.

Modern control operations against preventable maladies are usually aimed only in a nebulous way at the complete removal of the disease from the community involved. Not until about a decade ago did the concept of widespread malaria eradication become acceptable as a practical proposition. For there are formidable differences between the requirements of malaria control and those of malaria eradication.

Some of these differences are as follows: malaria control aims to reduce the morbidity as much as feasible, but eradication must completely stop malaria transmission: control is usually limited to readily accessible areas of greatest economic or political importance, but eradication must include all localities where transmission takes place, regardless of location, degree of endemicity, or of economic and political importance: control projects often have no foreseeable end, but eradication schemes have a planned end point: case finding and

reporting, with investigation of individuals and verification of positives by blood examination, have a relatively minor place in routine malaria control, but are of primary importance in eradication schemes: in control, one stresses the accomplishments, but in eradication it is the estimate of what remains to be done that is most important: good results are acceptable in control programs, but perfection is the goal of eradication.

That these strict requirements for malaria eradication are not beyond attainment is clear from results already recorded in several nation-wide malaria eradication projects, some of which will now be described.

### SOME NATION-WIDE MALARIA ERADICATION PROJECTS

Convincing demonstrations of large-scale malaria eradication by residual spraying have been made in wide areas of the Americas, Europe, and Asia but not yet in Tropical Africa. Some sizable experiments are in progress in West and in East Africa but it is too early to speak of nation-wide eradication in tropics where *gambiae* is the vector. Certain other areas, such as the Arabian Peninsula, Ethiopia, Borneo, and New Guinea, are not yet ready for eradication projects except on a trial basis. India, East Pakistan, Burma, Indonesia, and the countries of Indo-China also present difficult problems although the evidence is that all of the vectors involved, except *A. sundanicus* in Java, are amenable to DDT residual spraying. Thus far, *A. sundanicus* has responded satisfactorily to Dieldrin spraying in the few areas where it has been tried. In large countries, such as India and Indonesia, malaria eradication by stages is feasible, taking one major area after another.

Venezuela was first to capitalize on the combination of a sound epidemiological understanding of malaria and a full appreciation of the possibilities inherent in DDT. Under the guidance of Gabaldon, there was formulated in 1945, the earliest national project that from its inception was designed to eradicate malaria from an entire country by DDT residual spraying. DDT was in limited use by civilians in 1945, but no other scheme up to that time had announced "total malaria elimination" as its goal. In Venezuela, where malaria was the most important health problem, the disease has now been reduced to relatively minor incidence. There are still foci of endemicity and the chief vector, *A. darlingi*, that had all but disappeared by 1954, reappeared in some areas in 1955, but the disease is on the way out. It now does so little harm that lay officials are inclined to think that the campaign is over. This belief is, of course, dangerous. Only when there has been no natural transmission of malaria for three consecutive years, with at least three more years of surveillance, can an eradication project be ended.

Malaria eradication in Italy, including Sicily and Sardinia, is now almost complete. No malaria deaths have been recorded since 1948. The total numbers of primary infections were 9 in 1953, 6 in 1954, and 5 in 1955. Of the 5 cases reported in 1955, two were quartan malaria transmitted by blood transfusion: the others were vivax, presumably contracted by local mosquito transmission. These data are in marked contrast to the 303,057 cases with 8,407 deaths in 1919 and the 411,602 cases with 386 deaths in 1945. The eradication project in Italy was started in 1946 by the late Professor Missiroli and it is a notable monument to his genius and to the skill of Italian malariologists. No larviciding at all for malaria control has been done in Sicily or on the Italian mainland since 1946. In Sardinia, there was a special experiment in 1946-1950 to find out if vector eradication would be feasible and more satisfactory than vector control. The transmitting mosquito, *A. labbranchiae*, was not extirpated in spite of intensive efforts that included both larvicides and adulticides. Malaria, however, was eradicated. The per capita cost was four times greater in Sardinia than on the mainland, where it was 50 cents per year, with equally good results.

In the United States, the objective of malaria eradication was set in 1947 and this goal has now almost been attained, largely because of the addition of DDT residual spraying to the malaria control program. Inspired by Williams, and under the guidance of Andrews and his colleagues in the United States Public Health Service, some 6 million DDT residual sprayings had been made by 1950 and malaria had been dealt a final blow. The Health Service, in a careful survey in 1954, could find only 8 cases of primary indigenous malaria in the entire country. This was in marked contrast to 1915 when eleven southern states reported over 780,000 cases. Malaria had been declining in the country for half a century or more, due to a tremendous amount of drainage that materially reduced the incidence of the principal vector, *A. quadrimaculatus*; also to wide use of household pyrethrum sprays;



to widespread larviciding programs by county and state health departments; to a considerable migration of gametocyte carriers northwards to cities where there could be no transmission; and to other factors, social and economic. But the addition of DDT residual spraying upset what promised to be a prolonged period of light malaria incidence with occasional areas or years of localized epidemics.

Ceylon was highly malarious for centuries and no other disease did so much damage to its economy. A nation-wide scheme was set up in 1946–1947, and, under the late Doctor Rajendram, succeeded in 1955 by Doctor Gunaratne, it is coming close to its goal. Of the total population of about 8.39 millions, some 3 millions live in areas where malaria has been endemic and where the DDT residual scheme was applied. As a result of the spraying, the numbers of malaria cases reported dropped from 3.4 millions in 1940 to 7,317 in 1955, and the morbidity rate fell from 574 per thousand in 1948 to 0.85 per thousand in 1955. Infant mortality rates from all causes have been halved. An efficient malaria surveillance service has been established and spraying has been stopped in some areas. There is no reason to doubt that within a relatively few years Ceylon will have eradicated its ancient enemy.

There are also notable residual spraying projects in Brazil, British and French Guiana, Greece, Madagascar, Iran, Thailand, Formosa, the Philippines, Indonesia, and elsewhere. More and more eradication projects are being planned on a regional basis. For example, the Pan American Sanitary Bureau—World Health Organization Regional Office, in co-operation with the United Nations Children's Fund, ICA, and the Governments of the countries concerned, has mapped out and started an eradication project that includes all of the Americas and West Indies. Another regional scheme embraces the Near East,—Iran, Iraq, Turkey, Syria, Lebanon, Jordan, Israel, and Egypt.

### RESISTANCE TO INSECTICIDES

The development of anopheline resistance to residual insecticides has aroused considerable apprehension among malariologists although there is general agreement that malaria eradication projects now in progress can be completed successfully in spite of this new handicap. If, however, the concepts of eradication were abandoned, there is every reason to believe that long continued malaria control by the residual toxicants would not be possible.

At present well-marked physiological resistance to DDT is known in *A. sacharovi* in Greece and Lebanon, in *sundaicus* in Java, and in *stephensi* in Saudi Arabia. Strong resistance to Dieldrin, gamma-BHC and chlordane is known in *A. gambiae* in Nigeria and *quadrimaculatus* in the United States. There is some evidence that *A. superpictus* and *maculipennis* are developing DDT resistance in Greece. In each of the above cases, only circumscribed and relatively small areas are involved. In many countries no evidence of any resistance whatever has appeared after 6 to 11 years of spraying. But evidence suggests that the foci of resistance may be expected to multiply. There has also been noted a behavioristic resistance, manifested by an avoidance of contact with the toxicant, in *A. albimanus* in Panama, *gambiae* in Mauritius and *sundaicus* in Java, possibly a natural character in the last two.

It appears from recent studies by Macdonald and colleagues on a colony of Dieldrin-resistant *A. gambiae* that the gene of high resistance in the parent mosquitoes remains relatively unchanged and is distributed in the progeny by simple Mendelian laws of inheritance so that a "resistant" population consists of three groups of individuals: susceptible, hybrid, and highly resistant.

Obviously, there is great need for more thorough and extensive field studies and also for fundamental research work in the biochemistry and genetics of the resistance problems.

### INTERNATIONAL COOPERATION

The 1956 malaria budget of the World Health Organization is about \$309. thousands. This will be spent to provide world-wide co-ordination of malaria programs, and to make available technical advice and a few field demonstrations. The 8th World Health Assembly in Mexico City in 1955 strongly endorsed the principle of malaria eradication. To the World Health Organization should go great credit for its part in the amazing acceleration in world-wide malaria control and eradication since 1946. The Malaria Section, under the

skilled guidance of Doctor Pampana, and the five WHO Regional Offices have stimulated nation-wide projects, demonstrated the feasibility of residual spraying in many areas, provided fellowships and training courses, organized regional malaria conferences, and fostered basic research.

The Pan American Sanitary Bureau with its own funds, and also as the American Regional Office of WHO, has had a vital part in pushing forward the attack on malaria in the Americas and it is now coordinating the continent-wide eradication scheme. In 1956, its malaria budget totals some \$193,000.

The United Nations Children's Fund (UNICEF) has provided millions for national malaria control and eradication projects from the first years of its existence, realizing clearly the importance of this disease to infants and children. The 1955 malaria budget was about \$3.2 millions and in 1956, UNICEF is allocating some \$7. millions for malaria projects. This money is not used for personnel but for insecticides, equipment, and transport.

The United States Government has had a vital part in the world-wide attack on malaria. In 1955, in addition to its contributions of \$3.35 millions to WHO, \$6.645 millions to UNICEF, and \$1.32 millions to PASB, the United States, through ICA, spent some \$8.51 millions for malaria control and eradication projects in 15 countries. In 1956, ICA will spend more than \$14. millions for malaria eradication throughout the world. The Division of International Health of the USPHS, under Williams succeeded by van Zile Hyde, has been responsible for policy decisions, for providing trained personnel, and for much of the great success of malaria control projects.

Canada and the United Kingdom and many other countries have actively supported the malaria policy of WHO and UNICEF. The UK has allocated to malaria control some of the funds of various Colonial Development Schemes and has also financed the Colonial Insecticides Committee which has carried out a long series of research studies that have shed much light on the mode of action of the residual toxicants.

National Governments of the non-Communist world, excluding Africa, spent an estimated \$31.2 millions in 1955 for the attack on malaria, and in 1956, the estimated expenditure is \$43.7 million. The total amount being expended by all national and international agencies in non-Communist countries, excluding Africa, in 1956 for malaria control and eradication, is estimated at some \$66. millions.

### NEED FOR ENTOMOLOGICAL RESEARCH

In the early years of this century we were primarily interested in taxonomy and distribution of anophelines and the discovery of vectors of pathogens. Then, up to the period of World War II, the ecology of larvae assumed major importance and was widely studied. We have now come to a time when we must direct much more attention to the physiology of adult vectors, to anopheline genetics, and to the vital statistics of anophelines. Clearly, there is need for more information about flight habits and longevity of certain vectors. Even more useful would be an understanding of the genetic and physiologic principles of resistance so that we would not have to move in the dark when a vector begins to be less susceptible to DDT or to Dieldrin.

Today there are new tools available in cytogenetics and the use of radioactive isotopes but they have had surprisingly little use so far. Since the first observations of the value of these isotopes in mosquito studies by Bugher and Taylor (1949) and by Hassett and Jenkins (1949) there have been relatively few reports about this technique in the study of mosquito dispersal and range, resting habits, food preference, longevity, biochemistry, genetic changes and other aspects of mosquito ecology and physiology.

Not only are there immediately pressing problems but experience would indicate that other basic and at present unforeseeable questions will arise. There is vital need for forward planning of the scientific aspects of the world-wide malaria eradication program, especially as regard entomological research.

### CONCLUSIONS

The eradication of malaria from entire countries, regions, and even continents is certainly practicable today, both financially and technically. But success will depend on a still greater development of the international spirit, on the exercise of unusual vision,



courage, and steadfastness by the professional malariologists in charge of nation-wide projects; on strong administrative support by government officials, and, above all, success will come only if there is maintained a solid underlying foundation of concomitant basic research, especially in the field of malaria entomology.

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# *Anopheles gambiae* Giles (Diptera, Culicidae) and its Control by Residual Treatment of Houses

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## ABSTRACT

Limited to work in East Africa with which I have been personally connected this includes: 1. A brief outline of the life-history and importance of *A. gambiae*. 2. Influence of aspects of mosquito behaviour; exophily and endophily, host selection and how it varies in different places, frequency of feeding, resistance to insecticides. 3. Influence of insecticidal factors; formulations, size of particles, adsorption by mud. Different insecticides and their uses. 4. Measurement of efficiency of control. Adult catches in houses, outside searching and box traps, mortality measurements by new emergencies, pregravid and delayed sporozite rates, use of trap huts. Application to Taveta-Pare malaria control scheme.

## 1. INTRODUCTION

This is not a comprehensive survey of the subject, but is limited to points of interest from work in East Africa with which I have been personally connected.

*A. gambiae* is one of the only two vectors of malaria of any importance in Africa south of the Sahara and in many areas it is by far the more important of the two, maintaining intense malaria infections which may exceed 200 infective bites per person per annum. Although it is often considered almost a domestic mosquito and it is true that its breeding is often greatly increased by man's activities, it is notable for the variety of situations in which it will breed, and its larvae may be found in any reasonably clean water which is not fast running or heavily shaded. In some areas, however, the breeding taking place in small temporary sunlit pools is by far the most important. Although the adult is generally found mostly in human habitations it has been found in large numbers in the uninhabited Bwamba forest in Western Uganda and in, for example, the peculiar circumstances of Mauritius, it is found to a large extent in animal shelters rather than in houses. There is, of course, much variation in habit from place to place according to the changing conditions, but it may be said that the "normal" behaviour of an *A. gambiae* adult female throughout the warmer part of its range is feeding in a house on man during the night, remaining in the house for about 24 hours then leaving to lay its eggs and returning to feed again the next night, giving a 48-hour cycle (Hocking and MacInnes, 1948; Gillies 1954a; Holstein, 1954).

## 2. INFLUENCE OF MOSQUITO BEHAVIOUR

*A. gambiae* is generally an endophilic mosquito, sometimes 100% so, but even then activation by small doses of DDT may cause it to give up resting indoors. (In some highland malarial epidemic areas, however, this alone may cause interruption of malarial transmission because of the low outside temperatures during the night).

In some areas, for example the Gonja Valley area in the Pare district of Northern Tanganyika, a high proportion of *A. gambiae* feed on cattle and most of these rest outdoors. There are still large numbers of newly fed mosquitoes to be found in morning catches in houses and almost half of these have fed on man and half on cattle.

In Mauritius a certain amount of crepuscular outdoor feeding on man takes place but most *A. gambiae* feed on cattle and rest in cattle sheds (Halcrow, 1955).

These three cases are obviously quite different from the point of view of control by insecticides and the *A. gambiae* in Mauritius have in fact survived insecticidal treatment of all the buildings in the island over a period of 3 or 4 years, which treatment eliminated the other vector *A. funestus* (Dowling, 1950).

The main question in the Gonja type position is whether or not the zoophilic mosquitoes are behaving as an independent population and the evidence which I will go into below suggests that in the Gonja Valley they are not.

The frequency with which a mosquito returns to a house for food obviously affects the chances of it picking up a lethal dose of insecticide, but as this frequency is bound up

with temperature and hence the length of the intrinsic cycle of the malaria parasite it does not in fact make much difference in practice. What really matters is the number of chances there are of killing the mosquito before it becomes infective to man.

In Western Sokoto, Nigeria, *A. gambiae* has developed a marked resistance to insecticides after about one year's spraying with Dieldrin. The pure strain of resistant mosquitoes from this area is 800 times as resistant to Dieldrin and 40 times as resistant to BHC as a susceptible strain but as yet shows no resistance to DDT (Davidson, 1956). This capacity for the development of resistance is obviously of the greatest importance in control measures.

### 3. INFLUENCE OF INSECTICIDAL FACTORS

(i) Insecticides can be sprayed onto walls of houses as oil solutions, oil-water emulsions or suspensions of water dispersible powders and it was early discovered that to obtain a deposit on the surface of a porous substrate such as a mud wall it was necessary to use a suspension from which the insecticide particles are filtered out as the water soaks into the wall. On impermeable surfaces oil solutions are more suitable (Hadaway and Barlow, 1949).

(ii) The size of the particles of insecticide left on the wall surface has a considerable influence on the effectiveness of the deposit, the median lethal dose of insecticide increasing rapidly as the size of the particles increases from 10 microns to 100 microns. This is because the smaller particles are more easily picked up and retained by the tarsi of the mosquito (Hadaway and Barlow, 1951a, 1952).

(iii) Another factor which greatly influences the effectiveness of insecticides on mud is the adsorption by the mud wall of dry insecticide crystals on which Hadaway and Barlow have done a considerable amount of work (Hadaway and Barlow, 1951b, 1955). In laboratory tests on purified mud blocks, 10 micron crystals of DDT, BHC or Dieldrin, on active mud, disappear in a day or so at low humidities and a mosquito can then stand quite safely on the blocks treated with Dieldrin or DDT but will still be killed on the BHC block by its fumigant action. This phenomenon is greatly affected by humidity. At low humidities the disappearance is very rapid but the insecticide does not go very far into the mud, forming a layer just under the surface. At high humidities it goes in more slowly, but more deeply. Increasing the humidity of an already treated block causes the insecticide to move in both directions and actually increases the toxicity to mosquitoes on the surface. This finding is supported by field trials in which kills have increased following rainy periods (Burnett, 1956a; Hocking, 1947b).

Larger crystals persist for much longer periods on active mud surfaces but are not so effective in killing mosquitoes.

In practice the adsorption of insecticide by mud does not appear to be so vital as might be expected. This is partly because of the proportion of non-mud surfaces in any hut—doors and windows and frames of wood, and roofs of grass or reed thatch. In most muds there are impurities such as sand grains, pieces of straw, etc., which are impermeable to insecticide, and some muds are comparatively inactive. In commercial wettable powders there is a considerable range of sizes of insecticide particles and hence a comparatively long life on an active mud and during that time a continuous supply of small crystals. There is also the possibility of some kill of mosquitoes in a hut by air borne particles or vapour of insecticides (Davidson and Burnett 1952).

(iv) There was considerable doubt as to whether malaria transmission in the hyperendemic areas of tropical Africa could be interrupted by the residual treatment of walls of houses with insecticide. Before commencing a large scale field experiment therefore, we spent 5 years carrying out trials in experimental huts with different insecticides on different wall surfaces to try to determine the insecticide most likely to succeed. These trials were carried out at Taveta, in Kenya, in huts made of the same structure as typical African huts but mosquito proof except for the eaves and fitted with window traps to catch mosquitoes leaving.

The result of the trials undertaken first by Davidson (1953) and then by Burnett (1956b) were briefly as follows: DDT if applied at 2 grams per square metre in a wettable powder with small particles (about 10 microns) will give 60–70% kill of all mosquitoes entering the hut for about six months even on an active mud, but the kill with DDT cannot be made greater than this. BHC wettable powder at 0.2 grams gamma isomer per square metre will give 100% kill of mosquitoes entering the hut for only a month or so at high

temperatures and on not very active mud, but for six months or more at the same dosage on an active mud. Dieldrin at 0.4 grams per square metre in a wettable powder, will give a very high kill up to a year on mud of low activity, but for only 3 or 4 months on an active mud. At 0.8 grams per square metre it will give a very high kill for up to a year in huts with any type of wall surface.

These dosages are those required actually on the wall and because of wastage due to fall out and drift of particles the nozzle dosages used should be 25–30% greater (Burnett and Woodcock 1955).

#### 4. MEASURING THE EFFICIENCY OF CONTROL

The normal routine method of assessing the adult population of a malaria vector mosquito is by hand catches or spray catches in houses (Hocking 1947a). Because of the ability of some insecticides to drive *A. gambiae* outside with a sub-lethal dose this method will give only negative evidence in a control scheme. That is to say that if mosquitoes are caught in the house it means something is wrong, but if none are caught it does not mean everything is right.

In our present large-scale field experiment, which is being carried out in the Pare district of Tanganyika and the Taveta district of Kenya we are, therefore, supplementing this method by others, as follows:

(i) Routine outside searches in fixed natural resting places and in trap boxes. The wooden frame trap boxes are 3 feet by 2 feet by 3 feet high, have plastic gauze sides, back and top and the open front is covered except for a small gap by a removable dark cloth. These boxes are covered in soil in groups of ten and have produced several hundred *A. gambiae* per group per day.

(ii) Use of trap huts as described in 3 (iii) above. These are erected in pairs throughout the area, one is sprayed and the other is not, and they are slept in by bait Africans.

For ten days daily records are kept of the dead mosquitoes on the floor, live mosquitoes in the hut and of mosquitoes in the window trap with their immediate and delayed mortality. This routine is carried out on each pair of huts every month.

(iii) Mortality measurements. The efficiency of transmission of malaria parasites by a mosquito depends on the maintenance of a sufficiently long average mosquito life for a high percentage of the population to become infective. If the average age of a mosquito population falls drastically it is obvious that its ability to transmit malaria has been seriously interfered with even if its numbers are maintained. Dr. M. T. Gillies of the E. A. Malaria Institute at Amani has been working with us on the Pare malaria control scheme on ways of measuring this effect.

If the mortality rate is constant throughout the life of a female mosquito this mortality rate and hence the average life of a population can be determined from the delayed sporozite rate (i.e. the proportion of mosquitoes at any one time that have lived long enough to become infective compared with those that have only lived long enough to become infected (Davidson, 1954)). But apart from its assumptions of a constant mortality this method depends even more vitally on the death rate of the mosquito not being affected by infection by the malaria parasite.

Dr. Gillies found that the first gonotrophic cycle in *A. gambiae* required 2 blood meals whereas later cycles only require one, therefore it is only nulliparous females that can be found in the half way or pregravid stage in the morning—all others are either newly fed or fully gravid (Gillies, 1954b). Hence, the percentage of young females and a measure of the average age of the population can be determined. He has recently discovered that newly fertilized females contain a visible mating-plug in the oviduct and that this is a more precise character by which to determine the young female percentage.

In the Taveta-Pare malaria control scheme, the mosquitoes caught in outside box traps (as in 4 (i) above) have all had their latest meal on cattle, but the young female percentage in these mosquitoes went up strikingly after the first spraying of the houses which suggests that this population was being killed. This probably means that these *A. gambiae* are facultative feeders taking some meals on cattle and some on man according to opportunity.

The second spraying in the Taveta-Pare control scheme has now been completed, the house-haunting *A. gambiae* population is very low and the malaria parasite rate in small



children has already fallen considerably, so that, unless serious resistance to Dieldrin develops, the outlook is promising.

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# Malaria-Control Problems in Vietnam<sup>1</sup>

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## ABSTRACT

*The problems of inaccurate records, transportation, DDT residual treatments, national customs, insecurity, and complexity of anopheline species are discussed in so far as they are related to the reduction of malaria in Vietnam. There are 22 known species of Anopheles in the region. Of these two are effective vectors and four others are partially effective.*

## INTRODUCTION

The problems encountered in efforts to control malaria in Vietnam are many and varied. Some of them are concerned with lack of trained personnel. Such problems can be resolved, although that requires time. Others are more complicated because they are very closely associated with the very life of the people—their habits, their ideology, and their security. These latter problems do not yield easily to western ideas of organization and management—and they should not be subjected completely to our kind of logic. Rather, we foreigners must bend to unaccustomed lines of approach and emphasis. These latter problems— indefinite, in some cases even unmentionable—are no less vital to us who are undertaking a disease-control project than problems having to do with the insect vector itself.

## THE PROBLEM

In 1950 the immediate problem was one of reducing the incidence of malaria, as part of an American aid impact program in Indochina. The area of responsibility was reduced by the Geneva Conference on July 20, 1954 to the part of Vietnam below the 17th Parallel, and a further reduction in area resulted on November 1, 1954, when separate USOM offices were set up in Cambodia and Laos.

At present there are about 12,500,000 persons living in Free Vietnam, 1,000,000 of whom have recently taken part in one of the largest mass migrations in history. The incidence of malaria is spotted in intensity; in general, the highest rates of infection occur in the northern mountainous areas of the country.

In accord with recommendations made by the Second Asian Malaria Control Conference, at Baguio, Philippines, November 1954, our goal has been changed to eradicating the disease before the appearance of anopheline resistance to insecticides. There is reason to believe that this goal can be reached, with a reasonable number of ifs. If the areas are secured, if we succeed in building up an effective working organization with definite responsibilities, and if adequate supervision continues, then we should effect eradication within 3 to 6 years in those areas.

Scientists of the Pasteur Institute in Saigon long ago interested themselves in the malaria problem and have made many local surveys throughout Indochina. They have invariably reported higher rates of infection than we have found during the past 5 years. Although malaria is a reportable disease in Free Vietnam, the diagnosis and the method of reporting are too inaccurate to yield valid figures. For example, not only are nonmalarious cases sometimes recorded as malaria but an individual who appears at a clinic several times and is always diagnosed as having malaria is included in the records each time as a new case.

In July 1955, with a staff of Vietnam technicians newly trained in the Philippines to make malaria surveys, we began our first nationwide survey. Within the past 12 months we have completed collecting basic data in 15 of the 34 Provinces of Free Vietnam. These 15 Provinces are the most malarious. They comprise three-fourths of the country's total area, but they have a population of not more than 4,000,000 out of the national total of about 12,500,000. The population of each of the 15 Provinces surveyed and the plasmodic and splenic indices are given in Table I.

<sup>1</sup> The data and observations in this paper are those of the author, and do not necessarily reflect the policy of the International Cooperation Administration.

<sup>2</sup> Retired Nov. 1, 1956.

TABLE I—Incidence of Malaria in each of 15 Provinces of Free Vietnam as Determined by a Survey Conducted in the Period July 1, 1955—May 1, 1956.

Province	Population	Plasmodic index	Splenic index
		Per cent	Per cent
1. Quang Tri	204,000	7	22
2. Thua Thien	552,000	3	67
3. Quang Nam	700,000	2	12
4. Quang Ngai	358,000	2	28
5. Kontum	85,000	11	37
6. Pleiku	160,000	1	8
7. Binh Dinh	900,000	5	37
8. Phu Yen	310,000	3	43
9. Darlac	93,000	17	17
10. Khanh Hoa	156,000	5	14
11. Phan Rang	106,000	9	25
12. Haut Donnai	60,000	6	16
13. Binh Thuan	248,000	1	13
14. Baria	60,000	3	6
15. Phu Quoc	10,000	1	5

Of the total estimated 12,500,000 population of Free Vietnam we are presently protecting about 3,500,000 by means of residual DDT and Dieldrin sprays. An additional 2½ million need this protection; giving it to them has been prevented largely by security problems.

CLIMATE

Free Vietnam, as a whole, is hot and humid, like the other countries of the Indochinese peninsula. Within it, three regional climates can be differentiated.

I. The climate of Saigon and Southern Vietnam is typical of the entire delta of the Mekong River up to Cap Padaran, and West of the Annamese chain on up to the 17th parallel. It is characterized by a definite change of the monsoons, the regularity of its rainy season, and the constancy of temperatures. The year is divided into three seasons: First, the summer monsoon, May to October, which is very wet and therefore has somewhat reduced temperatures; second, the winter monsoon, November to mid-February, which is dry and relatively cool; third, the season at the end of the winter monsoon, mid-February to April, which is dry, hot, and very humid. The annual average temperature in Saigon is 26.9° Centigrade. It has a maximum of 32.0° and a minimum of 23.2°. Annual rainfall averages 1,974 millimeters in Saigon. The humidity averages 80.9 percent. Typhoons are rare in this area.

II. The climate of Central Vietnam, east of the Annamese Chain from the 17th parallel to Cap Padaran, is characterized by four seasons: A dry and relatively cool season from February to April, more humid than that of Saigon; a dry and hot season from May to July; a rainy and hot season from August to October; and a rainy but cool season from November to January. The annual average temperature of 25.1° Centigrade in Hue, 25.5° in Tourane, with maxima of 29.3° in Hue and 29.5° in Tourane and minima of 21.6° and 22.5°, respectively. Annual rainfall averages 3,452.9 millimeters in Hue and 2,165.6 in Tourane. The humidity averages are 86.7 percent and 82.8 percent, respectively. Typhoons occur seasonally in Central Vietnam, along the coast North of Quinhon. They begin toward October and cease almost entirely in December. They are usually accompanied by heavy rainfall.

III. The P.M.S. is subject to the same precipitation as the delta region of South Vietnam. Its temperatures, however, are noticeably lower than on the plains. The annual average is 20.8° Centigrade in Djiring, with a maximum of 26.4° and a minimum of 16.9°. In Djiring the average annual rainfall is 2,091.6 millimeters, the average humidity 82.9 percent.

HUMAN GEOGRAPHY<sup>3</sup>

Of the estimated 12.5 million persons living in Free Vietnam an overwhelming majority are Vietnamese—racially, culturally, linguistically. There are, however, sizable alien and

<sup>3</sup> Data provided by Mary Slusser, anthropologist.



ethnic minorities, together totalling nearly 1.9 million persons. Alien minorities include Chinese, 700,000; Cambodians, 400,000; Indians, Pakistani, and Malays, 29,000; and French, 7,000. Ethnic minorities consist chiefly of some 735,000 tribal Indonesians, sometimes miscalled "Moi" (this is an objectionable term meaning "savage" in Vietnamese), and the closely related Cham; they include a few hundred tribespeople refugees.

The Vietnamese are by preference lowland dwellers. Though settled on the chain of small coastal deltas in Central Vietnam in a density averaging 1,000 persons per square mile, they are more sparsely settled on the great multiple deltas of South Vietnam. There the average number of persons per square mile is 400 elsewhere than on Saigon-Cholon, where it is 250. In the past few decades only, an increasing number of Vietnamese have established themselves in mountain areas—chiefly as plantation labor and truck gardeners. This movement has resulted from improved communications, increased malaria control, and pacification of the tribal Indonesians.

Approximately 9/10ths of the Vietnamese are subsistence rice farmers, intensively working small parcels of land by traditional hand methods. In addition to rice they raise fruits, vegetables, pigs, and poultry and buy or catch fish.

The rural Vietnamese are village dwellers, tending to occupy compact settlements in Central Vietnam and along the canals and waterways of South Vietnam. Vietnamese farmhouses are of wattle and daub or of straw; both types are built flush to the ground or, at



Fig. 1. A group of mountaineers in Vietnam. These people are of Indonesian ancestry, aloof from Vietnamese culture and customs. The tall man at the right is my interpreter assistant, Dao Luong Ngoc, a pure Vietnamese. Our driver, extreme right, is also pure Vietnamese.

most, raised a foot or two above surrounding ponds and flooded ricefields on platforms of tamped earth. Windows, if any, are small; interiors tend to be dark, smoky, and—in the rainy season—damp. None have running water or interior plumbing; few boast more than the most rudimentary outdoor sanitary facilities. Human waste and such garbage as is not fed to pigs is used for fertilizer or disposed of in rivers and streams or in stagnant ponds near the houses. These water sources also serve for laundering, bathing, and, where there are no dug wells, for drinking water.

The Indonesians in Vietnam live almost exclusively in the mountains, where they have taken refuge under the pressure of the expanding Vietnamese, inhabiting the hitherto uncoveted malaria-ridden highland slopes and plateaus. The tribal Indonesian mountaineers, though composing about 6 percent of the population of the Republic, are in large majority culturally unassimilated. They are passive toward or withdraw from the national life, and are distrustful and antagonistic toward the Vietnamese.

These Indonesians depend for their subsistence largely on shifting cultivation of dry rice and various other crops plus hunting and fishing; a few, where conditions permit, are sedentary wet-rice farmers. Woodland fields and garden plots are prepared by the wasteful slash-and-burn technique. This usually leaves the soil exhausted at the end of about 3 years, whereupon the farmer abandons the land and begins the same exhausting and wasteful exploitation of new lands elsewhere. The Indonesians are in no sense nomads, however; although they are frequently forced to reestablish their villages nearer to increasingly distant fields, their wanderings are in a limited area and often bring them or their descendants back to the original point of departure.

Indonesian villages are extremely variable, ranging from a huddle of sordid huts in the jungle to an orderly aggregation of prosperous-looking houses sheltering several hundred persons. Houses are characteristically built of woven bamboo and thatch and raised high off the ground on piles; the N'ong tribe, however, build directly on the ground. Small, single-family houses and longhouses sheltering 10 to 30 families each are equally popular, the choice depending on the social organization of the tribe in question.

The cambodian minority, composing 3.3 percent of the population of the Republic, is concentrated in the western provinces of South Vietnam, where, as the original settlers, they have been bypassed and surrounded by the westward-pushing Vietnamese.

Cambodian life in Vietnam, except for minor details, mirrors Cambodian life across the border. Almost all the Cambodians in Vietnam are rural settlers depending on rice, farming, gardening, and fishing for their livelihood. A very few have settled in Saigon and other South Vietnamese towns. The majority live in all Cambodian villages apart from the Vietnamese and have little or no contact with the latter aside from commercial and official interchange. Cambodians in Vietnam prefer the ancestral stilted type of house. A few have adopted Vietnamese-style clothing, at least for daily wear.

Other minorities tend for the most part to urban dwellers and share many of the material aspects of the Vietnamese urban pattern of living.

### PROBLEMS OF SECURITY

Problems of security face anyone entering certain areas in Free Vietnam. The hazards result from Vietminh ambush, from anti-Government activity, or from hostile mountain people. Although mountain tribes are usually friendly, in some of them human sacrifices occurred during the time I was living in the country.

Because of subversive activities, villages treated with DDT may be out of bounds when ready for further treatment a few months later. This problem is rife throughout Indochina, as throughout Burma, Malaya, and Indonesia.

The mountain tribes, by the way, are suspected to have high malaria rates and this must be considered in a malaria-eradication program. Although they have few contacts with Vietnamese, sooner or later, in trading timber, rice, and fabrics, they remain in the lower villages often and long enough to spread gametocytes among the local population.

### ACUTE PROBLEMS OF TRANSPORTATION

Throughout Free Vietnam there are large areas served by no road larger than a foot-path. In the wet season these trails may be covered by several feet of water. Guides and



guards are needed by anyone using these "highways" to remote tribal villages, many of which are 50 miles from the end of a passable jeep road. There are literally dozens of villages in the mountain areas where the only approach to the homes of 2,000 or 3,000 people is over a series of single logs crossing a stream or, as often as not, not quite crossing it.

In some places the problem of transporting equipment and insecticidal materials is solved only by using elephants or hand labor. Along the coastline from southern Vietnam to the 17th Parallel, a 250-mile section of the highway has been wrecked to such an extent that traffic cannot possibly move faster than about 10 miles an hour. A few years ago the Vietminh systematically removed sections of the hard surface about 20 inches wide and 2 or 3 inches deep at car-length intervals, and rain has eroded the resulting shallow trenches of soil. Also, bridges over all the coastal rivers were destroyed, with the result that all traffic must be "poled" across the rivers on pontoons. An hour is required to cross one such river, and at sundown the service is discontinued. Some effort is now being made to repair this highway, by hand labor, but the work is progressing slowly. Present travel, therefore, is reduced to a minimum.

For the purpose of making a malaria survey I entered a remote area in Central Vietnam, adjacent to the Cambodian border, by 3 days' travel on elephant back. Guides had to be hired to show the way, and guards had to be kept on watch during the night as a precaution against tigers. Several rivers had to be forded. The dry terrain up and down steep valleys was rocky and rough. The trail led through great patches of open woodland where the grass was higher than an elephant's eye, through almost impassable tangles of vines and other jungle growth. The jungle paths were crisscrossed by wild elephant trails, and signs of a variety of game birds and animals were everywhere. According to the local villagers, my associate and I were the first Americans to go into the area—indeed, the first outsiders, including foreign hunters, to go in within a period of 3 years. At the end of the trail we found two small villages of about 150 persons each. In one family of 5 children we found three blood smears positive for malaria.

We hope the recent availability of a 75 percent water-wettable Dieldrin product will make the residual treatment of this area easier.

### PROBLEM OF TRAINED PERSONNEL

The problem of available personnel trained for technical projects is almost paramount in underdeveloped countries. In Vietnam civilian doctors number less than 200, engineers are few and usually are found only in the larger centers of population, and representatives of the related professions are few or absent.

In February 1955 I visited the southern tip of Vietnam only a few days after the departure of the Vietminh. In this area of 100 square miles, at the center of which lies the village of Camau, there were about 300,000 persons without a doctor. Operation Brotherhood went into the area at the same time, and its services were very valuable and much in demand.

Vietnam has but one trained entomologist. He is in the Agricultural Services. After 6 years of malaria control, we have a few other persons in the Malaria Control Services who can accurately determine both larval and adult forms of anophelines. Our technicians are not broadly trained.

Within the past 18 months we have sent 8 persons from Vietnam to the Malaria Training Center at Tala, in the Philippines, for 10 weeks and have sent 15 to the Malaria Training Centre in Southern Taiwan for 8 weeks. In each course the student group was divided into three parts; one to learn how to make spleen examinations and diagnose blood smears; a second to learn the taxonomy and habits of *Anopheles* mosquitoes; and a third to learn how to formulate and apply residual insecticides. This method of teaching malaria-control personnel, unsatisfactory and weak though it may be, makes it possible to start a program within a comparatively short time with fairly capable personnel. Because English is not generally spoken, there is the critical problem of translating quantities of directions into Vietnamese. This is complicated at times because of the deficiency of the Vietnamese language in modern scientific terms. In such instances, French is used. Instruction in malaria-control practices in Vietnamese was given for the first time, in Hue, within the past year.

Sometimes I have had to use two interpreters to make myself understood—one to put my English into French and one to put the French into the language of the mountain tribes.

In such instances one's time is reduced by one-half or more and one always runs the risk of inaccuracies in interpretation.

We have found it difficult sometimes to get approval from the Vietnamese Government for out-of-the-country training for one reason or another, in spite of the fact that USOM is prepared to pay the expenses involved. This problem is met, mostly, by repeated effort.



Fig. 2. A mountaineer's guard station, where he keeps watch to protect his crop from marauding animals. Cords running from the shelter to the limits of the field produce a noise when pulled. Such shelters, remote, inaccessible, and expensive to treat because of time to reach them are a key to satisfactory malaria control in the mountains.

### PROBLEMS RELATED TO CUSTOMS OF THE PEOPLE

Two of our problems are closely related to the customs and habits of the people.

First, among the mountain tribes in which malaria is severely epidemic there is an agricultural practice that complicates our control procedures. Villages of a few dozens or few hundreds of people may be miles removed from the end of a jeep road and accessible only by footpaths through jungles and up steep mountain grades and primitive bridges over rivers. Extending in every direction from these villages are patches of cultivated land—some of them so far away from the village that the owner goes to them only at intervals and remains several days, alone or with his family. In the center of each of these cultivated patches of upland rice or corn is an elevated shelter where the owner spends the night and from which he and any other occupants can frighten away marauding wild pigs, deer, monkeys, and elephants. These shelters must be treated with residual insecticides even though the treatment—which, itself, may require a total of only 5 minutes—necessitates half a day's journey. We have found the treatment of such shelters a key to the control of malaria in the mountain villages. Furthermore, after using one of these remote patches of cultivated ground for 2 or 3 seasons, the owner may leave and take up occupancy at a still more remote place.

The second complicating factor in the habits of the people is a common custom of giving no names to children until they are of school age. In making and checking a parasitic



and splenic index of an infant or child, the lack of an identifying name sometimes offers a real problem.

### FINANCIAL PROBLEMS

The problems having to do with finances are not those involved in inadequacy of funds; rather, they are concerned with procedures for getting the money to the end point. The steps are many and tortuous, and sometimes there are leakages en route.

One situation that seems peculiar in the light of our western ideas of logic existed during the war—and still persists in isolated instances. The DDT spray teams were greatly depleted by military drafts of manpower. When a team member was drafted, the Malaria Control Budget continued to pay him, to the extent that his military pay fell short of his Malaria Control Services salary. Many of our spray teams were reduced by one-third or even by one-half, but we could not fill those vacancies because we were paying for services not available to us. Consequently, we simply could not carry out the projected program.

Another special financial problem makes it difficult to fix a definite salary budget. Technicians and others doing the same kind of work do not necessarily receive identical salaries; the salary scale is based on one's family responsibilities. A foreman with 8 children, for example, receives far more than a colleague having only 2 or 3 children and may even receive more than his supervisor if the supervisor has only 2 children. Therefore, in order to determine a salary budget one must learn first the size of each employee's family.

### ANOPHELINE PROBLEMS

Our collections of adult *Anopheles* within houses and larval *Anopheles* in the field totaled 22 species. These are: *aitkeni*, *barbirostris*, *hyrcanus*, *sinensis*, *jeyporiensis*, *aconitus*, *maculatus*, *karwari*, *maculipalpis*, *culicifacies*, *lindesayi*, *philippinensis*, *vagus*, *subpictus*, *kochi*, *tessellatus*, *minimus*, *sundiacus*, *niggerimus*, *gigas*, *leucosphyrus*, and *fuliginosis*.

The abundance and distribution of these species varies considerably and are dependent upon the topography, season, and kind of water available. The most important fact is that *A. minimus* and *A. jeyporiensis*, both effective vectors, represent more than 80 percent of the catches within homes in areas where they occur. *A. sundiacus*, an effective vector in brackish water along certain sections of the coast, is the most numerous within homes in those sections. Elsewhere in Vietnam and throughout the extensive rice lands of Southern Vietnam, *A. vagus*, an inefficient vector, is most abundant.

With regard to anopheline breeding in Vietnam, we may roughly distinguish six separate environmental zones:

1. Sealevel coastal area, with lagoons of brackish water, where the flora and climate are typically maritime. *A. sundiacus* breeding in the brackish water and *A. subpictus*, *sinensis*, *vagus*, *tessellatus*, *barbirostris*, and *maculipalpis*, in various kinds of fresh water. Endemic indices ordinarily low, splenic index from 4 to 10 percent.

2. Sealevel to about 35 feet elevation, where the water is fresh and most of the land is given over to rice production. *A. sinensis* and *vagus* are the most abundant anophelines, with occasional specimens of *niggerimus*, *aconitus*, *barbirostris*, *tessellatus*, *minimus*, *kochi*, *philippinensis*, and *subpictus*. Endemic indices are slightly but definitely higher than in group 1. Splenic index 5 to 20 percent.

3. Elevation 35 to 60 feet. Agriculture includes rice terraces, coffee, tea, tung, and rubber plantations. There are many fresh-water streams, and correspondingly much greater numbers of *A. minimus* and *sinensis*. *A. aconitus*, *vagus*, *barbirostris*, *fuliginosis*, *maculipalpis*, *philippinensis*, *tessellatus*, and *kochi* are present, but in comparatively small numbers. Endemic indices are higher, splenic index is 10 to 20 percent.

4. Elevation 60 to 1000 feet. This is a zone of rugged, heavily watered terrain, with plantations of rubber, tea, and coffee. *A. sinensis* and *minimus* are by far the most numerous anophelines. The other species present are *jeyporiensis*, *aconitus*, *vagus*, *maculatus*, *aitkeni*, *karwari*, *fuliginosis*, *maculipalpis*, *philippinensis*, *kochi*, and *tessellatus*. Splenic indices are as high as 35 to 50 percent in many localities.

5. Elevation 1,000 to 3,000 feet. This is a well-forested zone, heavily watered, mountainous, and with elevated plateaux. There are rice terraces in some parts. *A. minimus* is most numerous, *A. sinensis* almost as abundant. Species present in smaller numbers include *A. maculatus*, *jeyporiensis*, *aconitus*, *vagus*, *aitkeni*, *gigas*, *barbirostris*, *fuliginosis*, *maculi-*

*palpis*, *philippinensis*, *kochi*, *leucophyrus*, and *tessellatus*. Spleenic indices are usually above 50 percent.

6. Elevation over 3,000 feet. Very rugged terrain separated by deep valleys and heavily watered with torrential watercourses during much of the year. A zone greatly restricted in extent. Species present in about equal numbers are *A. gigas*, *lindesayi*, *sinensis*, *jeyporiensis*, and *maculatus*. Scattered individuals are found of *aitkeni*, *barbirostris*, *culicifacies*, *fuliginosis*, *philippinensis*, and *kochi*. The spleenic index ranges from 0 to 3 percent.

### SUMMARY

We believe malaria, although probably the number one disease in Vietnam, can be eradicated by means of an effective working organization in spite of unusual difficulties and involved working procedures.

# National Malaria Control Programme in India

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## ABSTRACT

The National Malaria Control programme in India was started in 1953 as a part of the health development programme of the first five-year plan for the country. The plan envisaged an operative phase of three years (which was later modified to five years) and a maintenance phase thereafter. The plan provided for the protection of 125 million only, out of the 200 million estimated to be at risk (estimated annual morbidity of 75 million and mortality of 0.8 million). The plan was extended to protect the entire population at risk, during the second five-year plan, the ultimate objective being the eradication of the disease by stages. The financial implications of the plans amounting to Rs 1151.68 Lakh (115 million) were met by the Government of India, the State Governments participating in the programme; American Aid and United Nations International Children's Emergency Fund, etc., transportation, equipment, and insecticides were made available by the latter organizations. Direction, co-ordination and assessment were allocated as central government's responsibility to be discharged by the Malaria Institute of India. The participating States were to be responsible for the execution of the programme, etc. (Jaswant Singh 1953).

162 Malaria Units fully equipped have been set up so far, each Unit being responsible for protecting one million population. The method of malaria control provided was to intercept the transmission of malaria by the application of residual insecticides in the dwellings (75% water-dispersible DDT) applied once, twice, and rarely three times a year depending on local conditions, using a total dosage of 200 mg. of technical DDT per sq. ft. of wall surface annually. During 1954-55 about 15.5 million houses were sprayed and 8 million lbs. of DDT was consumed. There has been a fall of nearly 19.4 million malaria cases in 1954-1955 alone as compared to 1953-1954. It would appear that on the basis of three days sickness per case and a daily wage of Rs 2 (two) the amount of money saved would be nearly 11.64 crores of rupees (Rs 110 million), very nearly the sum expected to be spent on the programme for the three-year period.

The concept of eradication will involve: (a) inclusion in the programme of all the areas, even those of very low endemicity; (b) speedy achievement of interception of transmission to avoid the risk of development of strains of mosquitoes resistant to insecticides; (c) development of sensitive indices to assess the degree of control, etc.; (d) surveillance teams to control any possible outbreak of the disease. "While it is true that the experience of a few nation-wide control programmes elsewhere in the world was available, for sheer magnitude, the variety of technical problems and the number of vectors involved, the National Malaria Control Programme of India remains to date unique and biggest in the world" (Rao 1955).

For details readers are referred to publications by Jaswant Singh (1953) and Rao (1955).

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## DISCUSSION

L. A. JACHOWSKI, JR. Has the malaria control program had any noticeable effects on other arthropod-borne diseases?

R. PAL. Yes. Some of the collateral effects have been in the control of plague and fly-borne diseases. The relief from nuisance of bed bugs has been another feature of the programme making it very popular among the public at large.

SERGIO BETTINI. I like to point out that in Italy (Sicily) we have exophilic anophelism (*A. labranchiae*), but without any malaria transmission. We have in Italy *Cimex lectularius* resistant to chlorinated hydrocarbons. Is there any sign of resistance in India?

R. PAL. Bed bugs have been reported to have developed resistance to chlorinated hydrocarbons recently in the State of Bombay.

D. R. JOHNSON. Is there any relationship between the India malaria control program and the India national filaria control program?



R. PAL. Both the programmes are the responsibility of the Malaria Institute of India in collaboration with the various States. The Indo-American aid by way of supplies of equipment and transport, etc. has made it possible. Both survey and control units have been set up in 13 States where filariasis is prevalent. Administration of filaricides, control of larvae and adults of vector species is contemplated.

D. R. JOHNSON. (a) What is the total number of persons protected against malaria in India at present by residual insecticides. (b) What is the total annual per capita cost of malaria control at present in India?

R. PAL. (a) Approximately 125 million. (b) About 8-as (10 cents) per capita.

R. LEVI-CASTILLO. Did you find any behavioristic type of resistance in *Anopheles* or any other type of resistance, because in Ecuador *Anopheles albimanus* has become exophilic after five years continuous DDT spraying in the coastal region?

R. PAL. The susceptibility of vector species to chlorinated hydrocarbons as applied is being continuously determined and behavior of these species to the application of insecticides. There has been no evidence so far either of physiological or behavioristic resistance. In fact some of the vector species have responded so well that after a year of application of insecticides adult mosquitoes are not readily available.

L. VARGAS. Dr. Pal, will you please give me information on the number of houses to be sprayed annually, on the average number of people living per house, on the average of surface to be sprayed per house, and the cost of spraying per house.

R. PAL. It was assumed that each village hut has 1000 sq. ft. area to be sprayed. The number of people were estimated as five. The numbers of houses sprayed annually are as follows:—1954–55 15.5 millions; 1955–56 22.3 millions. The cost of spraying per house is approximately Rs.2.50 (40 cents.).

R. M. GORDON. Some of us have had doubts regarding the ultimate success of an antimalaria scheme directed only against the adult. I congratulate Dr. Pal on the account he has given on the remarkable results achieved and some of which I observed for myself a few months ago when I visited Delhi.

# Contribution to the Knowledge of the *Anopheles* of Portuguese Timor as Vectors of Plasmodia

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## INTRODUCTION

During the months of November and December of 1955, and January, February and March of 1956, in the overseas province of Timor (Fig. 1) among other works we made a survey of *Anopheles* of the region. We caught 462 specimens in the larval stages and 599 adults. As it is a very mountainous region we studied the various species of *Anopheles* in relation to the altitude (Fig. 2).

We collected also the necessary material to study the preferred hosts and also samples of water of the larval breeding places to study its chemical composition. We also collected specimens of *Culicini* (not reported here).

The following species of *Anopheles* were caught:

1. *Anopheles (Anopheles) barbirostris barbirostris* Van der Wulp, 1884

We observed 11 larvae of this species from Lautem and Viqueque. They were found in pools of muddy water and in muddy ponds where buffalos usually bathe. The maximum

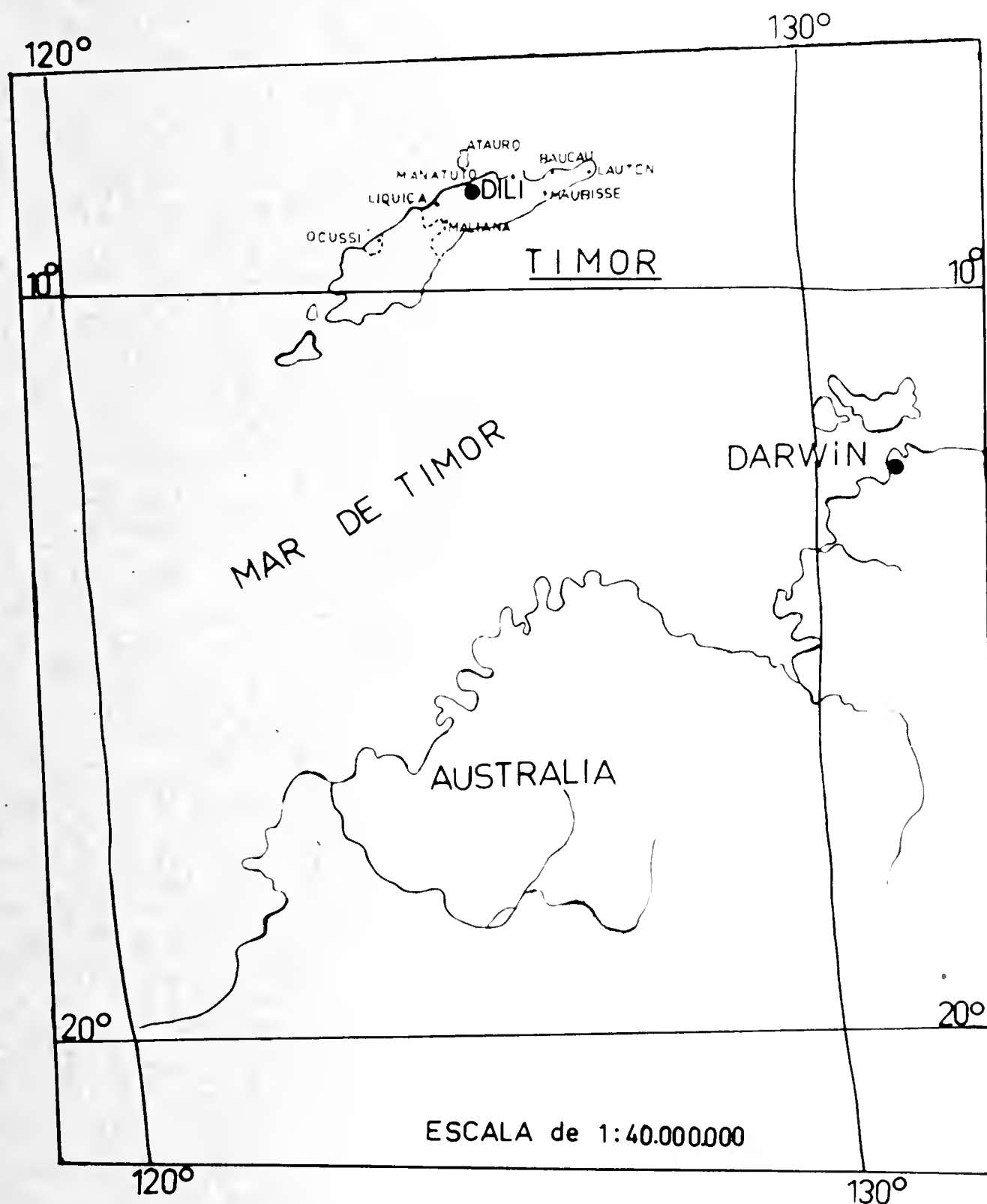


Fig. 1. Geographical location of Portuguese Timor.

altitude where we caught these larvae was 90 metres. We also caught 116 adults, of both sexes, near Baucau, Dili e Laleia at an altitude of 350 metres. We dissected 100 females from these places and observed an oocistic index of 4% and a sporozoitic index of 0.

2. *Anopheles (Myzomyia) aconitus* Donitz, 1902

We caught 4 larvae, 3 in Dili in small muddy puddles, and 1 in Maliana in the still water of the backwater of a small river. In contrast to the small number of larvae caught, we collected a large number of adults, especially in Baucau. We also caught some specimens in Dili. We collected a total of 110 specimens. The dissection of 100 females showed an oocistic index of 1% and a sporozoitic index of 0. The maximum altitude was 200 metres.

3. *Anopheles (Myzomyia) annularis* Van der Wulp, 1884

We caught only 1 larva, near Missão de Fuiloro, at about 250 metres altitude in a small pond with a sandy bottom. We collected 35 specimens in Baucau and dissected 28 females but none was infected by the plasmodia.

4. *Anopheles (Myzomyia) maculatus maculatus* Theobald, 1901

We caught 36 larvae in Baucau, Bobonaro, Ermera, Maliana, Maubisse and Ossu, in places that are far from the coast. We verified that these larvae do not appear in zones of low altitude but only at about 300 to 1.500 metres. We found them in small pools with gravel or sandy bottoms and in streams, ponds and ditches where the bottom was very muddy, lakes with muddy water (Lake Lapasapulo on the way from Bobonaro to Maliana) and small pools in cultivated fields. We caught 14 adults in Baucau at an altitude of between 290 to 340 metres. We dissected 7 females without positive results.

5. *Anopheles (Myzomyia) minimus* Theobald, 1901

We collected only 4 larvae, 1 in Baucau in a small pool with a sandy bottom and *Spirogira* algae, and 3 in Maliana in a small stream with sandy bottom without aquatic vegetation.

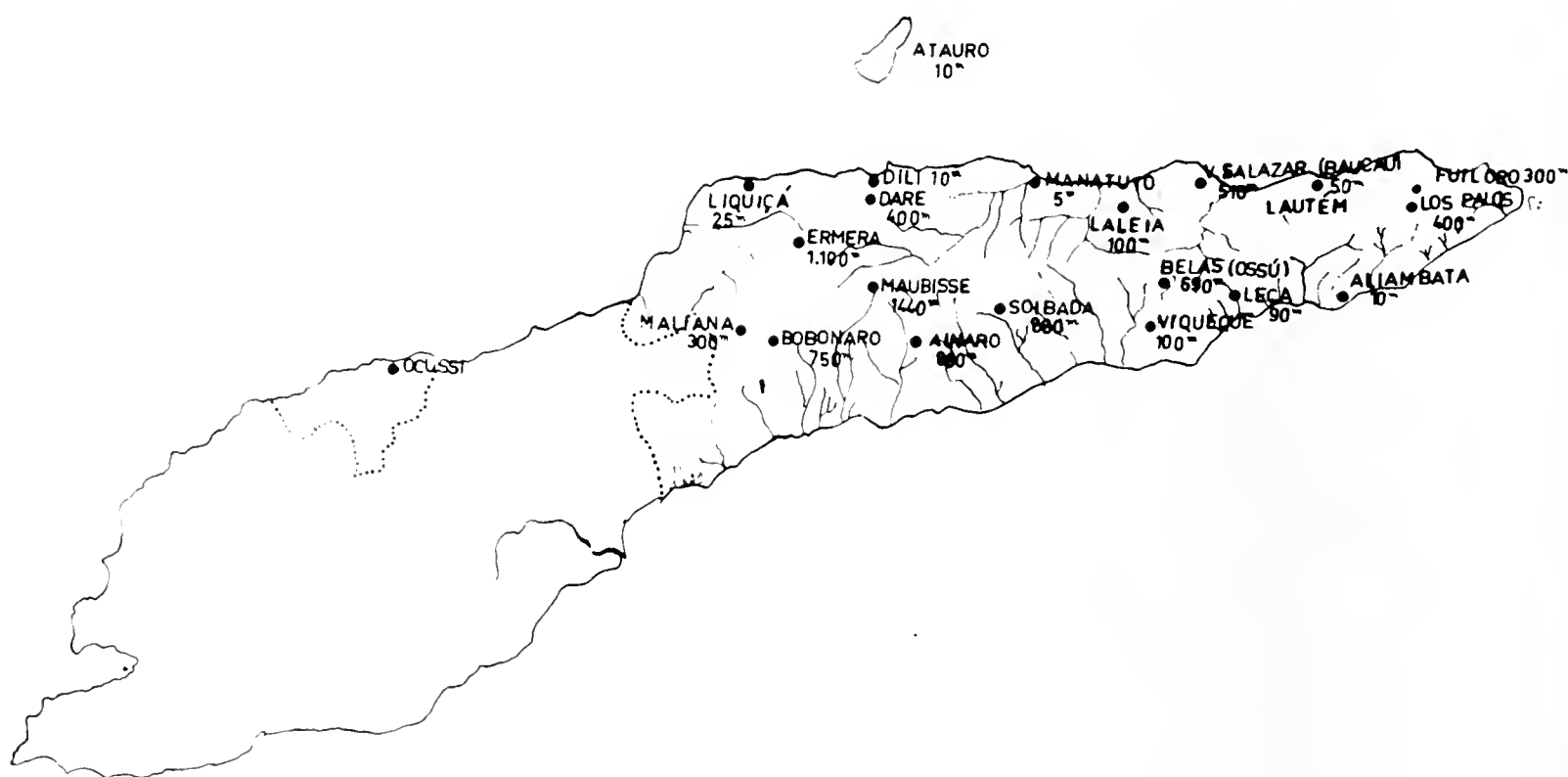


Fig. 2. Localities in Portuguese Timor where species of *Anopheles* were found.

6. *Anopheles (Myzomyia) subpictus subpictus* Grassi, 1899

We caught 27 larvae in Aliambata, Baucau, Dili, Fuiloro and Liquiçá. The most common breeding places were small pools with sandy or muddy bottom some with *Spirogira* algae, or small muddy puddles and in Aliambata we found 2 larvae in a small pool at about 15 metres distance from a source of crude petroleum. The breeding places were situated only at a low altitude of no more than 300 metres. We caught 166 specimens of this species in Baucau, Dili, Fuiloro, Lospalos and the island of Atauro, also at altitudes of no more than 300 metres. We dissected 100 females without positive results.

7. *Anopheles (Myzomyia) sundaicus* Rodenwaldt, 1926

We caught 105 larvae at Dili, Liquiçá, Lore-Irara, and the island of Atauro. The breeding places were nearly always near the sea. They were mainly small pools of rain water or water of other origin with sandy or muddy bottoms, sometimes with some *Spirogira* algae and often exposed to the sun. We caught 156 specimens most of them from Baucau, Dili and Liquiçá. We dissected 100 females and we found an oocistic index of 4% and a sporozoitic index of 0. The altitude was no more than 250 metres.

8. *Anopheles (Myzomyia) tessellatus* Theobald, 1901

We caught only 1 larva in Baucau, in a rice field.

9. *Anopheles (Myzomyia) vagus vagus* Donitz, 1902

It is the most widely distributed species or at least is the species which can most easily be found in the larval stage. We caught 273 larvae in Baucau, Bobonaro, Dili, Laleia, Lautem, Liquiçá, Lospalos, Maliana, Manatuto and Viqueque. They were found in small pools with sandy or muddy bottoms, in puddles in the footprints of buffalos and mainly in rice fields. They were found at altitudes from sea level to 740 metres. We were able to collect only 2 adults, 1 at Dili and the other in Oecussi.

TABLE I. Results of the Dissections of *Anopheles* Females.

Species	Number of dissected mosquitoes	Index	
		sporozoitic	oocistic
<i>A. barbirostris</i> barb.	100	0	4%
<i>A. aconitus</i>	100	0	1%
<i>A. annularis</i>	28*	0*	0*
<i>A. maculatus maculatus</i>	7*	0*	0*
<i>A. subpictus subpictus</i>	100	0	0
<i>A. sundaicus</i>	100	0	4%

\*Without statistical significance

## CONCLUSIONS

We could not visit all the places in the territory and our collection was made only during the rainy season, so we must make other surveys to complete this study. Nevertheless, we think that *Anopheles sundaicus* and *A. barbirostris* are the most important vectors of malaria in Timor, according to the results of dissections (Table I).

Concerning *Anopheles vagus vagus*, so very widespread in the territory, we suppose that it is not a vector of malaria there. Perhaps it is exclusively zoophilic as very few adult specimens were caught, either by day or night, in places usually frequented by man. More detailed study of the biology is necessary to arrive at definite conclusions.





# The South African Vector of Rift Valley Fever<sup>1</sup>

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## ABSTRACT

Rift Valley fever, a virus disease which affects mice, sheep, antelopes, cattle, and man, has been isolated in South Africa, Kenya, and Japan. It is now known that the mosquito, *Aedes* (*Ochlerotatus*) *caballus* Theobald, is a sylvatic vector of this disease. This vector is considered of international interest as it occurs throughout Africa, as well as around the Mediterranean Sea and is also known from Persia. Of special interest to Canada is the *Ochlerotatus* subgenus to which *caballus* belongs. Fifty of the 121 North American mosquito species are *Ochlerotatus*. Species of this subgenus are world-wide in distribution, and breed in temporary ground-pools formed by rain, melting snow, flood waters, and tidal marshes.

*Aedes caballus* overwinters in the egg stage. Not all the eggs respond to flooding simultaneously. Large numbers of larvae hatch at each flooding. One generation is produced if the water in a breeding-place becomes permanent. Adults are normally encountered near their breeding-sites except when the veld is wet. They bite during the day and rarely enter houses. Other ecological data are detailed. One epidemic caused a total loss of \$2,560,000 during one summer. The role of other mosquitoes in the transmission of Rift Valley fever is discussed. Epidemiological considerations based on findings in the Panveld of the Free State and Transvaal, Knysna with its summer and winter rainfall, and the role of irrigation schemes, as well as the possible spreading of the disease by cars, trains, and aeroplanes readily entered by *caballus*, are discussed.

## INTRODUCTION

Rift Valley fever (or enzootic hepatitis) is one of a large group of tropical diseases transmitted by mosquitoes, and affects mice, sheep, antelopes, cattle, and man. This disease has been isolated in Kenya, South Africa, and Japan. It has been experimentally determined by Gear *et al* (1955) that in South Africa *Aedes* (*Ochlerotatus*) *caballus* Theobald is the sylvatic vector of Rift Valley fever. This mosquito species is considered of international interest as it occurs throughout Africa, as well as around the Mediterranean Sea, and is also known from Persia.

Most of the Canadian mosquitoes belong to the *Aedes* genus. Out of a total of 121 North American mosquito species, 50 are members of the *Ochlerotatus* subgenus of *Aedes*. Species of the *Ochlerotatus* subgenus are world-wide in distribution, and breed in temporary ground-pools formed by rain, melting snow, flood waters, and tidal marshes.

## DISTRIBUTION

Records collected by our Plague Research Laboratory, the South African Institute for Medical Research, the Onderstepoort Veterinary Research Laboratories, other published data, and our own data (Steyn & Schulz, 1955) have been used to compile a distribution map of *caballus* in South Africa. It is known from the Transvaal, Orange Free State, Cape Province, South West Africa, Natal, and Basutoland.

During our work, high population densities of this species were found in the Panveld of the Free State, North-eastern Cape, the South-western Transvaal. The highest density was found near the Jan Smuts International Airport. A very extensive and complete mosquito-free zone has consistently been maintained around this airport. Only very weak populations were found in Natal.

It is concluded that *caballus* has a very wide distribution in South Africa. It is stressed that the larvae of this species has only recently been described (Hopkins, 1952), that most of the mosquito material consisted of larvae collected by field staff, that only one generation of *caballus* occurs in a breeding-place after every filling, and that the adults rarely enter houses. *A. caballus* will therefore probably be found in most loci and in every one of our 15 climatic zones in South Africa.

<sup>1</sup> Published with permission of the Secretary for Health, Pretoria.

## LIFE-HISTORY

## EGGS

It is assumed that, like other *Ochlerotatus* species, *caballus* lays its eggs on dry earth and singly.

Onderstepoort workers found that *A. caballus* and *A. lineatopennis* Ludlow show a definite preference for small or medium-sized pans or slight depressions forming part of the veld, i.e. marshy spots covered with grass and filled with rain-water. Eggs, ready to hatch, must be present in the dry breeding-places. Not all the eggs seem to hatch at the same time. A number appears to remain unhatched when the place dries up again, and perhaps the next contact with water will cause them to hatch. If this were not so, it would be difficult to explain the constant large numbers of larvae which hatch out every time the place is flooded, however short the intervening period.

Near the Jan Smuts Airport we once found that a very dense population of *caballus* adults was completely destroyed by a heavy shower of rain during one afternoon. The hatching of eggs as described above, therefore, ensures survival of the species. Our own extensive mosquito catches at Bapsfontein in the Transvaal, confirm the conclusion of Nieschulz *et al* (1934) that *caballus* overwinters in the egg stage.

## LARVAE

During the coldest part of the winter of 1953, after the second snow-storm in the adjoining Oudtshoorn climatic area, we found *caballus* breeding in vegetated furrows along cultivated lands, as well as in almost pure sea-water at Knysna. Breeding-places of this species usually contain vegetation. Permanent water is never utilized, and in common with species of the subgenera *Aëdimorphus* and *Banksinella* this species produces only one generation if the water in a breeding-place becomes permanent. *A. caballus* is also known from the saltpan area of the Orange Free State, where the pools are brackish.

The developmental period for *caballus* larvae during warm weather is 5–6 days, while the pupal period is 1–2 days. At Onderstepoort artificial flooding of breeding-places was used for the production of *caballus* supplies. Near Benoni I once found that more than 200 newly-hatched larvae could be collected with one dip of a ladle, 12 hours after the first spring rains.

Predacious *Aedes scatophagiodes* Theo., and *Culex tigripes* Grandpre larvae were the most important enemies of *caballus* larvae during the Onderstepoort investigations.

## ADULTS

We found no hibernating *caballus* adults during extensive weekly collecting and animal-baiting at Bapsfontein from 30 August to 15 October 1954, although this species was very common afterwards. At Knysna, in the South-eastern Cape, however, adults attacked humans at the beginning of August 1953. It is therefore concluded that along the Witwatersrand this species does not hibernate in the adult stage, while at Knysna it probably occurs throughout the winter.

As already mentioned, a very dense population of *caballus* adults was completely destroyed by a heavy shower of rain during one afternoon. Several successive heavy rain-storms at the beginning of spring, may therefore delay the persistence of dense *caballus* populations.

When adults hatched out in their breeding-places, they appeared simultaneously in thousands, so that, when walking alongside the edge of the water, at that time, large swarms of male and female mosquitoes were disturbed. *A. caballus* does not really fly around during the day, but on being disturbed and settling down thereafter on the body, immediately commences feeding. This species rarely enters houses. We have only occasionally found small numbers inside houses at Randfontein, in the Transvaal, and also in electric lampshades at Vaalhartz, Cape.

## BITING HABITS

At Onderstepoort only very few specimens were recovered from horse-baited traps within a few hundred yards of the breeding-ground which harboured thousands of adults. The traps therefore gave no accurate indication of the relative abundance of the mosquitoes present in the field.

In our experience *caballus* mostly bites during the daytime from early morning to 20 minutes after dusk. Lewis (1943) records *caballus* biting man in the dry season in Eritrea. We found it to be a very vicious biter of humans in the open. Its bites appeared to be as painful as those of *Anopheles coustani* Laveran, *Taeniorhynchus africanus* Theo., and *T. uniformis* Theo. At Randfontein, after successive wet spells, we found *caballus* in small numbers, biting humans more than 900 yards from its breeding-places. At Knysna it attacked humans in the open while it was raining and cold at the beginning of August. This mosquito bites human, horses, donkeys, cattle, sheep and mice. It is therefore important to determine whether it also bites birds, especially migratory birds which may introduce virus diseases.

### ROLE OF OTHER MOSQUITOES

Daubney and Hudson (1933) transmitted Rift Valley fever by the inoculation into susceptible lambs of *Mansonia fuscopennata* Theo., *M. versicolor* Edw., and *M. microannulata* Theo., at intervals extending to 9 days after a meal of infected blood.

Mulligan (1937) reports that a percentage of *Aedes* species collected in an infected area during an epizootic might harbour the virus, but all attempts at transmission of the virus by bite failed.

Smithburn *et al.* (1948) found the virus occasionally in *Aedes* (*Stegomyia*) *de-boeri* spp. *de-meilloni* Edw., *A. (Aedimorphus) tarsalis* Newst. group and in *Eretmapodites* species. Smithburn and his collaborators discovered that *E. chrysogaster* Graham is capable of transmitting the virus by bite from mouse to lamb, from mouse to mouse, and from lamb to mouse. They found that in *Taeniorhynchus fuscopennatus* Theo., and *T. uniformis* Theo. the virus may survive for some days but these species are not such good transmitters as *Eretmapodites* species. *Aedes aegypti* L. does not act as a vector.

Mulligan (*loc. cit.*) mentions that the inoculation to lambs and mice of *Aedes durbanensis* Theo. collected near the sheep bomas showed that a percentage harboured the virus.

It was found (Ann. Rept. S. Afr. Inst. Med. Res., 1953) about *Aedes caballus* and *Culex theileri* Theo. that: "Although both species harboured the virus only *O. caballus* could transmit it by biting. The first sylvatic and epizootic vector of the disease has therefore been identified. During the 1953 epidemic in the Luckhoff district it was noted that vaccination of the flocks of sheep with the egg-adapted neurotropic Smithburn strain of virus vaccine, prepared at the Onderstepoort Laboratories, effectively prevented the disease".

It was calculated that the total loss to the sheep and cattle industry, caused by the 1951 epidemic was 2,435,000 dollars (Schulz, 1951). Some farmers lost 100 per cent of their expected lamb crop during the 1955 season, and it was estimated that 1,000,000 lambs were lost during 1955 (Alexander, 1956).

*A. caballus* was present at Bultfontein during the 1951 investigations. The records show that this mosquito is known from every area where Rift Valley fever has been reported in South Africa.

### CAUSATION OF EPIDEMICS

The following considerations are regarded as the most likely explanations of epidemics:

1. *Panveld*: The panveld of the North-eastern Cape, Western Free State and South-western Transvaal experienced the 1950-51 epidemic. The 1953 epidemic occurred in the panveld of the Luckhoff area. The epidemics raged in summer and autumn as these areas have a summer rainfall. From 7-17 May 1953, when *caballus* was incriminated, we found no active mosquito breeding as there had been no rain for several weeks. The 1955 epidemic also occurred in the panveld area.

2. *Knysna*: At Knysna in the South-eastern Cape we found *caballus* larvae, pupae and biting adults at the beginning of August 1953. This suggests that at Knysna adults occur throughout the winter, and may carry the virus of Rift Valley fever through the winter.

Because of the adults found during winter and the active winter breeding, *caballus* populations may build up more often at Knysna with its summer and winter rainfall,

than in areas with only summer or only winter rainfall. In addition to this the winter breeding of *caballus* in almost pure sea-water, also indicates Knysna as a stronghold of this vector. Data on the distribution of Rift Valley fever anti-bodies in the different age-groups of cattle at Chalmsford, Knysna, suggest that this disease has been present on this farm for at least 8 years.

3. *Irrigation Schemes*: The Kamanasie, Kaffir River (Bloemfontein), Rietvlei (Modder River), and Vaalhartz Irrigation schemes fit in between the endemic Knysna and the epidemic panveld areas. The excess irrigation water often runs off into the veld and creates *caballus* breeding foci, throughout the winter, as well as long before and long after the normal summer rains. In the panveld of Luckhoff, the 1953 epidemic occurred in April, i.e. the end of summer, while at the Vaalhartz Irrigation Scheme the outbreak dated from 27 April, through May to 30 June 1951, i.e. early to midwinter.

### MECHANISM OF SPREAD

We have found that *caballus* enters trains, and readily travels by train. It also enters parked and travelling motor cars as well as parked, lighted aeroplanes. Infected *caballus* adults can therefore be mechanically spread from the endemic Knysna area and from irrigation schemes to epidemic areas. The disease can also be spread by infected humans and domestic stock during and after stock sales when animals are transported for several hundred miles. We have therefore recommended the immunization of all sheep and cattle in the Knysna area and at irrigation schemes in order to ensure the prevention of even a small scale further outbreak.

This mosquito may still be of further importance, as Steinhaus (1946) records that some significant information is available about the relationship between the virus of catarrhal fever of sheep and *Aedes caballus*.

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# Methods of Collecting Larvae and Pupae of Mosquitoes in Ecuador (Diptera: Culicidae)

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## ABSTRACT

*Field techniques for collecting larvae and pupae in the various kinds of breeding places found in Ecuador are described. The Levi-Castillo Box for transporting larvae and pupae, in use for more than 10 years in Ecuador, is redescribed. It offers great advantages in transporting larvae and pupae from breeding places to the laboratory. Techniques for operating the box and for collecting in each breeding place are described.*

## INTRODUCTION

The study of the Culicidae of Ecuador during more than 14 years of exploration has necessitated the adoption of methods suitable to the circumstances and situations encountered. This has placed great strain on our ingenuity. We have been able to develop for posterity methods that possibly are not new, but which are adapted to the different situations found in the many locations where we had to work.

The breeding places of the Culicidae in Ecuador can be divided into three kinds, as follows:

- (A) Natural breeding places at ground level.
- (B) Natural breeding places above ground level.
- (C) Adapted artificial breeding places.

These in turn are subdivided in accordance with the manner in which they are found. Some breeding places apparently natural are adaptations of the species as a consequence of the action of Nature and of the hand of man.

## MATERIALS AND METHODS

The equipment (Fig. 8) that we have used in our research on the breeding places of Culicidae has been designed to serve all circumstances and requirements and for this reason we describe it in detail so that colleagues may know the use of each piece and the reason for including it.

1. *Rubber bulb pipette*.—We always carry one of 100 ml. or 50 ml. capacity, to collect the water in breeding places that are difficult to explore, also to collect all the larvae and pupae and the micro fauna and flora of the breeding place, so that afterwards similar natural conditions are reproduced in the laboratory.

2. *Flashlight (type Eveready)*.—This is important for illuminating breeding places and observing larvae and pupae in them when light conditions are inadequate such as in tree-holes, bamboo knots, etc.

3. *Machete (Collins type)*.—This instrument serves to open pathways in the jungle and to cut tree trunks, bamboo stumps, and other uses in the tropical jungles. It is also a defensive weapon against snakes and other wild animals.

4. *Capillary dropper with amplified tip and iron enamelled pan*.—This is indispensable especially when studying Bromeliaceae and other jungle breeding places where the larvae and pupae must be separated from predators within the biotope, and such larvae as the *Toxorhynchites* have to be eliminated and placed apart. In the field and with a magnifying glass all larvae and pupae are separated according to genus into different breeding boxes to be transported to the laboratory to study the life-cycles complete, this being a normal procedure.

5. *The Levi-Castillo box for transporting live larvae and pupae*.—This special box (Fig. 9) designed by the author and originally described in 1950 was first used in 1945 and consists of the following parts:



Plate 1. Collecting mosquito larvae: Fig. 1, in epiphytic Bromeliaceae at La Reserva; Fig. 2, in pineapple plants, *Anana sativa*, at Rio Blanco; Figs. 3 & 4, in bamboo plants at Chilicay.

(A) Exterior metal box.—It must be a perfect square (we usually use a 9 x 9 x 3 centimeter box because it is more practical for our type of work). It must have holes in the bottom and in the cover top to allow water to drip and the larvae and pupae to breathe, respectively.

(B) Cotton pad.—This keeps the larvae and pupae moist in the box. It is vital to maintain such humidity if a long trip is intended.



(C) Gauze.—This keeps the larvae and pupae and microfauna and flora alive, and retains small leaves in decomposition and other residues that later serve to maintain the breeding place in simili-natural conditions. Also, the gauze prevents the larvae and pupae from becoming entangled in the cotton pad.

(D) Wooden frame.—This is made of pieces of wood that form the frame which holds in place the gauze and keeps it extended so as not to harm the pupae and larvae. The parts are movable so that the gauze may be taken out with the larvae and pupae and the natural food and debris.

6. *One litre flask with funnel (enamelled iron).*—This is used to collect the liquid that drips from the Levi-Castillo box into the funnel thus retaining the original water of the breeding places.

7. *Colorimetric paper ribbon (Du Pont).*—This is used in the field to measure the pH of breeding places when collections are made. We usually have a potentiometer in the laboratory for a correct reading of the actual pH of the water of the breeding place for ecological studies of habitats.

8. *Thermometer, Centigrade (or Fahrenheit).*—It is carried in a padded metal box to measure the actual temperature of the breeding place.

9. *Portable meteorological station ("Lufft").*—This small contraction is easily carried to record meteorological data. It consists of an altimeter, thermometer, hygrometer, and barometer.

10. *Compass and regional map.*—These are indispensable in finding breeding places and establishing their geographical location; also the direction of the sun with respect to the habitat and the extension of sun rays reaching breeding places.

11. *Indian hair sieve.*—This small sieve has been found essential for separating the larvae in the field and determining the species and possible predators.

12. *Metal kitchen spoon (white iron enamelled).*—Many sizes are carried for use in various breeding places. Some can be attached to long poles, consisting of sections that can be screwed together for use in breeding places in a lake or in a deep location; otherwise ordinary handles are used. These kitchen spoons are carried like golf sticks in different sizes and handles for different uses.

Adults are captured with nylon or light cotton bags, killed in chloroform bottles and placed in small metal boxes with naphthalene in the bottom in a piece of gauze to hold it in place. To eliminate the possibility of damaging or desquamating the adults, we use several coats of cellulose paper (Kleenex type), and never cotton because it damages the specimens by breaking the legs and desquamating them so that they cannot be identified.

We began our surveys by collecting adults. Now we collect them only if they are very numerous. Our usual procedure is to bring back the larvae to the laboratories or field stations, separate the lots of larvae and pupae in simulated natural conditions in iron enamelled pans and neutral glass containers. Larval and pupal skins are mounted and studied. The imagos are killed with the chloroform bottle after 24 hours and terminaliae dissected. Thus we have obtained the complete cycles of all the species of Culicidae found in Ecuador.

## MOUNTING METHODS FOR THE TROPICS IN USE FOR LARVAE AND PUPAE IN ECUADOR

The larval and pupal skins are placed in mounts specially developed in our laboratories in 1949. Adult terminalia are mounted in the same manner.

### POLYVINYL ALCOHOL METHOD (LEVI-CASTILLO TECHNIQUE)

Larval and pupal skins and also the male and female terminalia are placed overnight in a 10% NaOH solution to clear; neutralized for 1 minute in 1% acetic acid solution; washed for one minute in tap water; placed in staining solution (Gage-Komp stain) made as follows:

Put 0.5 grams of acid fuchsin in an Erlenmeyer flask with 25 cc. 10% HCL and 300 ml. distilled water. This is the stock solution. For use (12 hours) staining solution is diluted

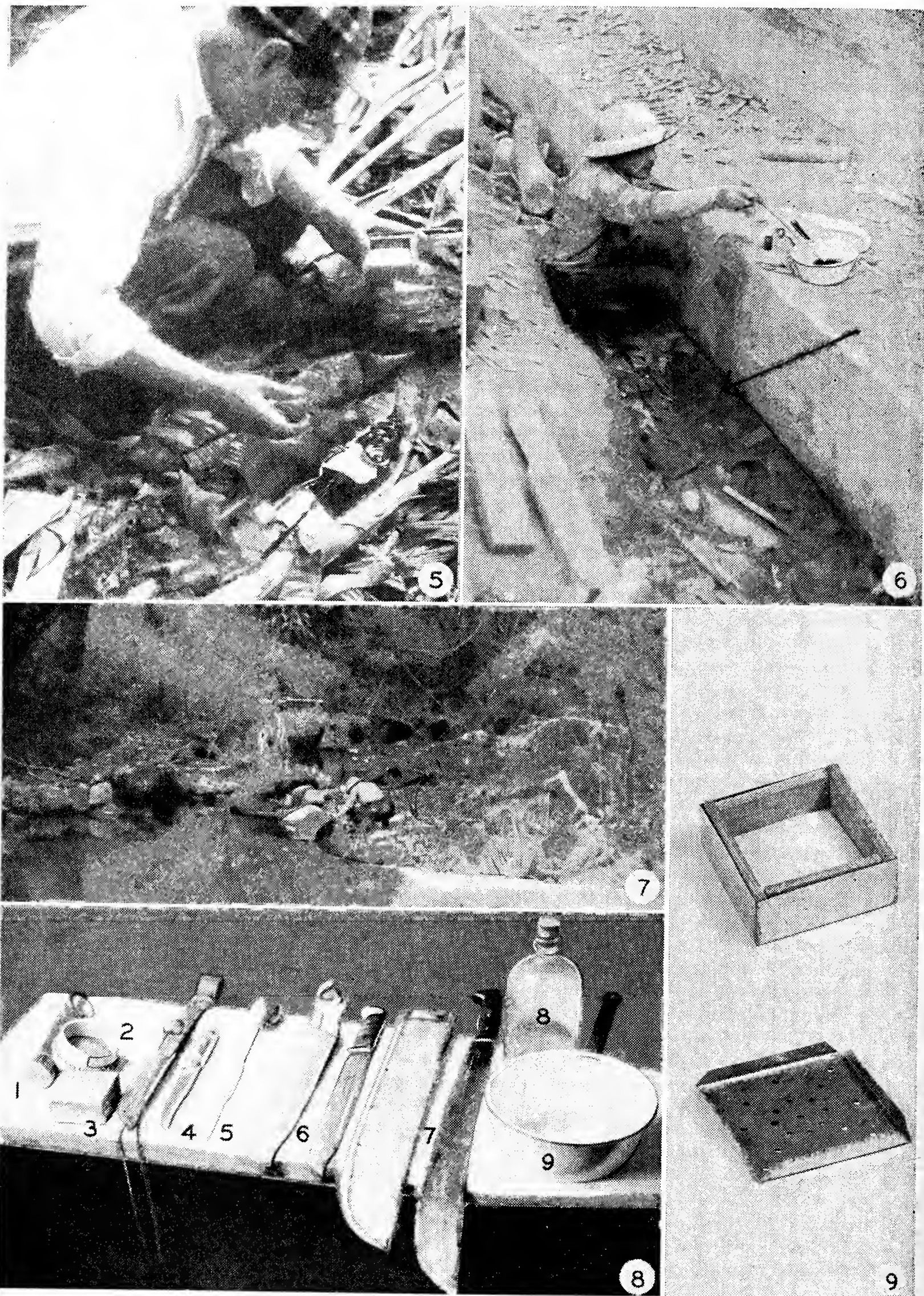


Plate II. Fig. 5. Collecting in banana leaves at Chilcales and, Fig. 6, in an obstructed irrigation canal at Bandurria. Fig. 7. Typical *Anopheles* breeding place in the coastal region. Fig. 8. Field equipment: (1) flashlight, (2) sieve, (3) L.-C. box, (4) knife, (5) pipette, (6&7) machetes, (8) graduate, (9) enamelled pan. Fig. 9. Levi-Castillo box.

1:5. Excess stain is cleared with lactophenol solution prepared with 10 grams of crystallized phenol in 8.2 ml. lactic acid, 10.8 glycerin, and 10 cc. of bidistilled water (pH 7). One drop is enough to clear the excess stain.



## POLYVINYL LACTOPHENOL LEVI-CASTILLO B-90-25-49

This mounting medium was developed in 1949 as the result of a search for a good mounting medium for the tropics, to preserve specimens and at the same time avoid molds and fungus that usually damage Canada Balsam preparations in a high humidity environment. "Elvanol" Du Pont was received in many viscosities at our laboratory through the courtesy of agents of that firm. All through 1948-1949 experiments were conducted until Type B Grade 90-25 Medium Viscosity was found to be the best; the others would easily crystallize and become turbid with time. Lactophenol is an old laboratory preservative, and we had in mind a combination of a clearing and a mounting medium; previously we experimented with phenol, oil of cloves, lactic acid and lactophenol, this last medium as described was found optimum for the combination.

Polyvinyl mounting medium solution was prepared by placing in an Erlenmeyer flask 40 cc. distilled water and heating it to 75°C. Then 10 grams of powdered Elvanol Du Pont Type B Grade 90-25 Medium Viscosity was introduced, stirring with a glass rod. In the beginning it is turbid but as soon as stirred it becomes crystal clear. To prepare *polyvinyl-lacto-phenol medium* we added 45 ml. lactophenol to the already prepared solution of polyvinyl when still hot, stirring rapidly with the glass rod; after it cools off the medium should be placed in a dark bottle because light turns it red by oxidizing the phenol. Lubkin & Carsten (1942) and Downs (1943) were the first to use this mounting method, but we never consulted these authors and our techniques were developed independently and adapted to our own use. We had idea of the existence of both research teams from Dr. P. A. Buxton who gave me the bibliographic data previously unknown to me; thus the methods were developed in our laboratories after 2 years of search and work with different mediums and plastics.

To dissect the pupae (cephalothorax) two No. 4 entomological pins are placed into 2 pieces of thin wood 4 centimeters long. These are placed in the center of two compact rubber balls (Sandoz balls) 3 inches in diameter; thus the whole hand moves the ball in the dissection under a Leitz binocular microscope (oculars G 18X; objectives 4X). The pins are sharpened into thin hair-sized microknives and dissection is effected under one drop of lactophenol.

After dissection, pupal skins and terminalia are mounted in a drop of polyvinyl-lactophenol medium between slide and cover slip. To eliminate air bubbles pressure is applied to cover slip and medium is viscous enough to hold the preparation. Dissection is effected in one part of the slide and pieces are transported by means of a steel dissecting needle bearing a drop of the medium by which the parts adhere to the needle point and are placed in the mounting drop. No dehydration or other methods are effected. Slides are dried for 48 hours in a dryer with calcium chloride to absorb water. The great advantage of the medium is that if the slide is improperly dried and parts dislocated, after 1 hour in a laboratory enamelled pan with tap water the plastic again becomes fluid, thus the slide is once again corrected and another drop of mounting medium added to regain consistence of mounting medium.

## TECHNIQUES OF COLLECTION IN ACCORDANCE WITH THE NATURE OF THE BREEDING PLACE HABITAT

## PINEAPPLE PLANTS, EPHIPHYTIC BROMELIACEAE &amp; ANTURIOS (ARACEAE)

If there is water in the plants (Figs. 1 & 2) the 100 ml. rubber bulb pipette is introduced and the water drawn up very slowly until the pipette is filled. It is then carefully removed from the plant with the index finger closing the tip while the pipette is raised to eye level and observed against the light for the movement of larvae and pupae, and emptied into a Levi-Castillo box. The thermometer is introduced into the leaves of the plant and the temperature recorded. A drop of the water is placed on a piece of colorimetric paper and the pH measured. A sample of the flower (if present) and leaf is taken to determine the plant species. All water is drawn from the "tank" of the Bromeliacea (Fig. 1) and then with a knife all the leaves and roots are cut off leaving only the "water tank". Each leaf is washed carefully and the plant disintegrated in an iron enamelled pan where the water from the Levi-Castillo box has dripped and been gathered. When all leaves have been washed the larvae and pupae are collected with the dropper and placed in the boxes and the water from the pan is collected in the funnel flask to be carried to the laboratory.

## BAMBOOS, BAMBOO STUMPS, TREE-TRUNKS, AND ROCK-HOLES

To collect in bamboo trees (Figs. 3 & 4) it is necessary to enlarge the holes originally made by insects and woodpeckers with a machete. This type of hole is so small that unless one is familiar with it, it can be passed undetected. A square hole is made to allow entrance of the pipette to take up the water as in the case of the Bromeliaceae. The procedure is more or less the same for tree-trunks and bamboo stumps. In the case of the latter, the complete bamboo pot is emptied into the Levi-Castillo box. The water in rock-holes is taken up with the pipette and the dripwater collected in pans. The breeding place is washed three times with the same water and afterward the complete liquid from the breeding place is taken to the laboratory. When larvae are captured in fruit covers (cocoa, coconuts, etc.), metal containers, tin cans, etc., great care has to be taken to bring to the laboratory (if possible) the whole fruit cover, to be used as a container of the liquids originally found there, so that conditions are reproduced in the laboratory as nearly natural as possible.

## FALLEN BANANA LEAVES

This type of breeding place (Fig. 5) is very common in banana plantations in the Coastal plain. After being cut the leaves collect humidity and rain water. To open up a breeding location the end of the stem is raised carefully with the tip of the machete, because the leaves may harbour small venomous snakes. The cartridge-like breeding place is then opened up. The water is brownish red with a pH of 6.0, 6.3, and 6.5 due to the presence of debris and decomposition of the leaf. The larvae are drawn up with the rubber bulb pipette and placed in the Levi-Castillo box.

## WELLS, LARGE COLLECTIONS OF WATER, SPRINGS AND POOLS

The iron enamelled metal kitchen spoons are used extensively in this type of habitat. In small pools the rubber bulb pipette has proved its worth.

## LABORATORY TECHNIQUES WITH LARVAE AND PUPAE

The Levi-Castillo boxes are brought to the laboratory and taken first to the insectaries where they are opened up. Sometimes adult mosquitoes escape into the insectary; that is one reason for not opening the boxes in the field after the collections have been made. When travelling long distances the boxes are stacked and held together with twine. Then, every day the water of the breeding place is placed in a wash tub and the box *wetted only to the cotton level*, to maintain humidity. Other water should not be used because it may harm the larvae and pupae. After the Levi-Castillo box is opened in the insectary and adults allowed to escape, the procedure is as follows:

(1) Place the water from the original breeding place in the iron enamelled cup (preferably white), where the insects will be bred and then (2) take apart the wooden frame holders and wash them one by one in the water. (3) Take the gauze between thumbs and index fingers and extend it into the water in the cup and move it up and down until all larvae and pupae and all debris and microfauna and flora from the breeding place are transferred into the cup by undulating hand movements. These techniques are simple and practical and we have used them with the best results ever obtained with all mosquitoes of Ecuador and especially with the phytothelms. The practicality of the Levi-Castillo box, 100% originally Ecuadorian in development, has been tested since 1945. With it we have never failed to bring back live larvae and pupae and to develop them under the best simulated-natural environmental conditions. This is the reason for bringing these methods to you, because you may use them elsewhere and in any country, and under any environmental conditions.

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# The Role of Mite Vectors in the Natural History of Scrub Typhus

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## ABSTRACT

*Rickettsia tsutsugamushi*, the agent of scrub typhus (mite typhus, tsutsugamushi disease) in man, is transmitted by the larvae (chiggers) of a number of species of trombiculid mites in an area of about five million square miles of land bounded roughly by Japan, India, and tropical Queensland. The major vectors of universal importance are *Trombicula* (*Leptotrombidium*) *deliensis* and *T. (L.) akamushi*. Their distribution, life-history, and biology are summarized. Three questions are discussed, concerned respectively with geographical distribution on a large scale, topographical distribution on a small scale, and seasonal distribution in time.

The geographical localization of mite-borne scrub typhus contrasts with the wide distribution of the tick-, flea-, and louse-borne rickettsioses. This is explicable by the hypothesis that scrub typhus has evolved in the broad area of evolution of both the genus *Rattus* and the chigger sub-genus *Leptotrombidium*, where extensive deforestation by man has encouraged dense populations of field rats infested particularly by the major vectors. The area of distribution of scrub typhus corresponds to that of the major vectors which have reached oceans and deserts barring further dispersal by wide-ranging hosts. The way in which the other rickettsioses differ is discussed.

There is experimental evidence for a primitive rickettsiosis (jungle tsutsugamushi) in trombiculids and their hosts, and its relation to the evolution of scrub typhus is described. The relationships between chiggers and their hosts are discussed in relation to the distribution of restricted 'typhus-islands' of infection which are grouped into endemic areas decided largely by topography and climate. The influence of climate is exemplified by perennial transmission in equatorial climates and by three types of seasonal transmission in monsoon and temperate climates. The number of life-cycles of the vectors completed per annum vary from about five to only one in these different countries.

## INTRODUCTION

Some of the most significant problems concerning the natural history of scrub typhus<sup>1</sup> are at the same time the most interesting, and I propose discussing three of these problems and taking the opportunity at the same time to summarize existing knowledge about the biology of the vector mites. The first of the problems is, why is the disease geographically confined unlike the other human rickettsioses which are represented in every continent (Fig. 1)? Second, why do restricted endemic foci of scrub typhus exist within endemic areas which themselves may be restricted (Fig. 2)? Third, why do accounts of the seasonal incidence of scrub typhus differ (Fig. 4)?

As a result of war-time investigations, two conclusions were reached (Audy 1949, Audy and Harrison 1951). The first conclusion was that scrub typhus is largely a man-made disease associated more than anything with dense colonies of field-dwelling rodents and two very closely related species of trombiculid mites, *Trombicula akamushi* and *T. deliensis*, the parasitic larvae of which transmit the infection. This is endemic scrub typhus as it is generally encountered by man. The second conclusion was that there exists a much more complex 'jungle-tsutsugamushi' cycle involving other chiggers and hosts. This cycle is still practically unexplored. Scrub typhus thus resembles plague and yellow fever in having an endemic or epidemic cycle into which man gets drawn, and a fundamentally different, more silent 'wild' infection which may rarely involve man. In each case, the endemic or epidemic cycle is a secondary development from the other.

The biology of the two major vectors of universal importance will be summarized first as a background to discussion of these cycles. A basic work is the manual of chiggers by

<sup>1</sup> Audy & Harrison (1951:372) consider that 'mite-typhus' is the official name of choice, being consistent with Megaw's classification of the rickettsioses by the major vectors, but 'scrub typhus' has become most familiar by usage and has practically replaced the original 'tsutsugamushi-disease'.

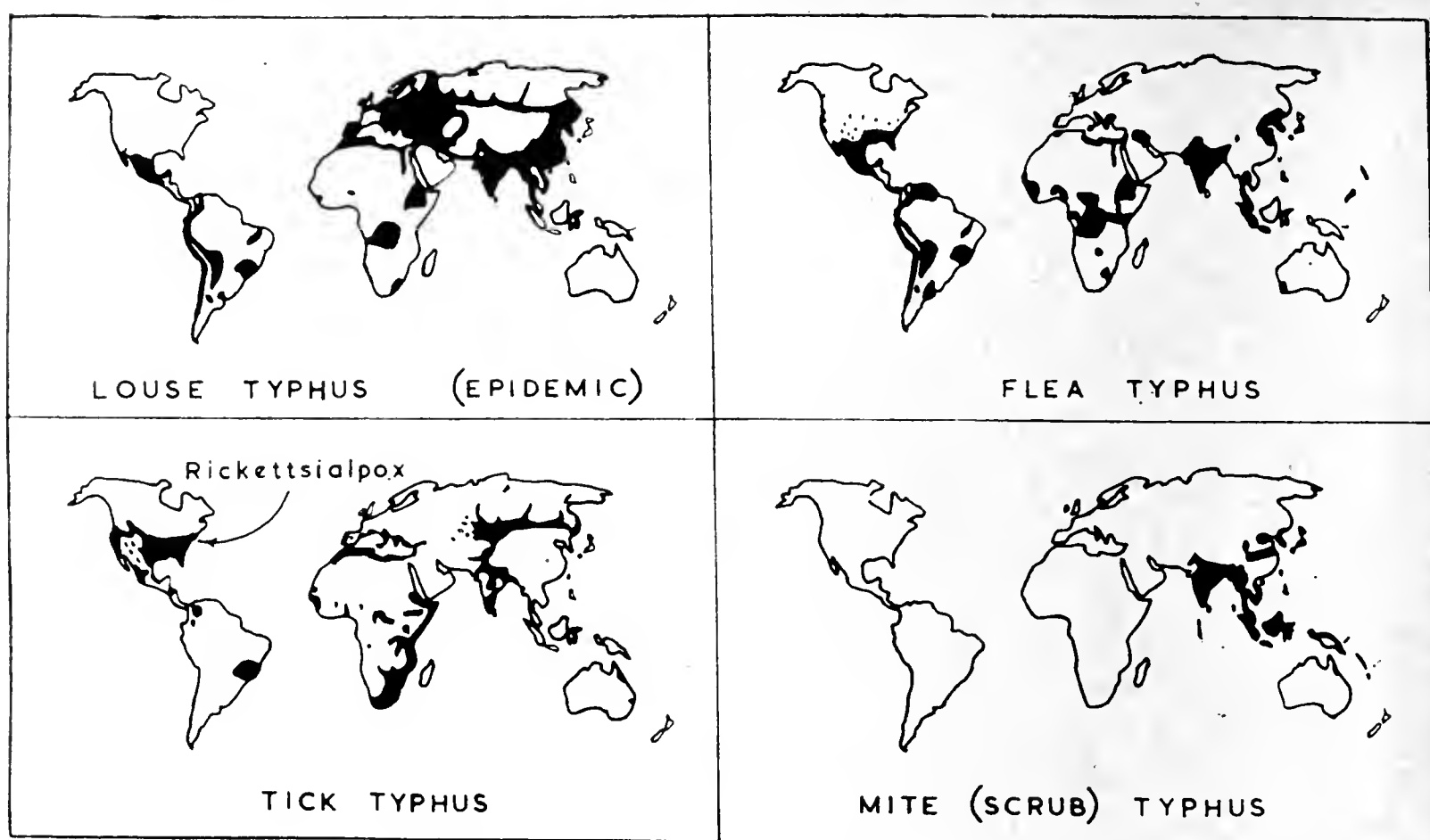


Fig. 1. Distribution of rickettsial diseases of man (from Audy 1958, with permission of the publishers of *British Journal of Clinical Practice*).

Wharton and Fuller (1952) which contains host and distribution lists, while a taxonomic review is given by Audy (1954b), and the life cycle of *T. akamushi* is described by Sasa and Miura (1953).

## THE MAJOR VECTORS *TROMBICULA DELIENSIS* AND *T. AKAMUSHI*

### GEOGRAPHICAL DISTRIBUTION

*Trombicula* (*Leptotrombidium*) *deliensis* Walch, 1922 (= *T. walchi* Wom. & H., *T. vanderghinstei* Gun., synonyms) is distributed over almost the whole Oriental region and extends from there to islands beyond New Guinea. Its presence has been confirmed in the following localities (among others) which roughly outline its range: Maldive Islands in the Indian Ocean (Radford 1946), Kashmir (Womersley 1952), Kunming in Yunnan (Millspaugh and Fuller 1947), Hong Kong (Audy 1954a: 34), the Philippines (Philip and Woodward 1946), the Bismarck Archipelago and New Hebrides (Kohls *et al.* 1945), the coastal strip above and below Cairns in tropical Queensland (McCulloch 1944), and Java (Gispen 1950a). It is absent from Guam (Wharton 1946) and has not been recorded from Formosa. Scrub typhus has been recorded from Diego Garcia (mid-Indian Ocean) and Espiritu Santo (New Hebrides), and it is safe to conclude that the vector in these places is *T. deliensis*, which has been distributed almost certainly by birds over stretches of ocean which must sometimes have been two or three hundred miles across.

*Trombicula* (*L.*) *akamushi* Brumpt, 1910 (= *T. fletcheri* Wom., synonym—*T. obscura* Wom. & H., usually quoted as a synonym, appears to me to be a distinct form which requires investigation) is less widely distributed and may have a palearctic centre of origin in the north-east. Recent locality records and places which roughly outline its known range are: Japan (Suyemoto and Toshioka 1955), Formosa, North Burma (Traub, personal communication), Philippines (Philip and Woodward 1946), New Guinea (Kohls *et al.* 1945), Borneo (Traub and Audy 1954), Malaya (Audy 1956b). It appears, at least for practical purposes, to be absent from India, South Burma, the area of Thailand round Bangkok, Hong Kong, possibly Sumatra and Java, Australia, parts of New Guinea, and many islands including Guam. In spite of intensive collecting it has not been found in central Korea, and has apparently not been recorded from South Korea, while it was rarely found in North Burma (Traub, personal communication). Malaya is one of the countries where both species occur together in considerable numbers, often in the same focus, and the small-scale features of distribution are discussed later.



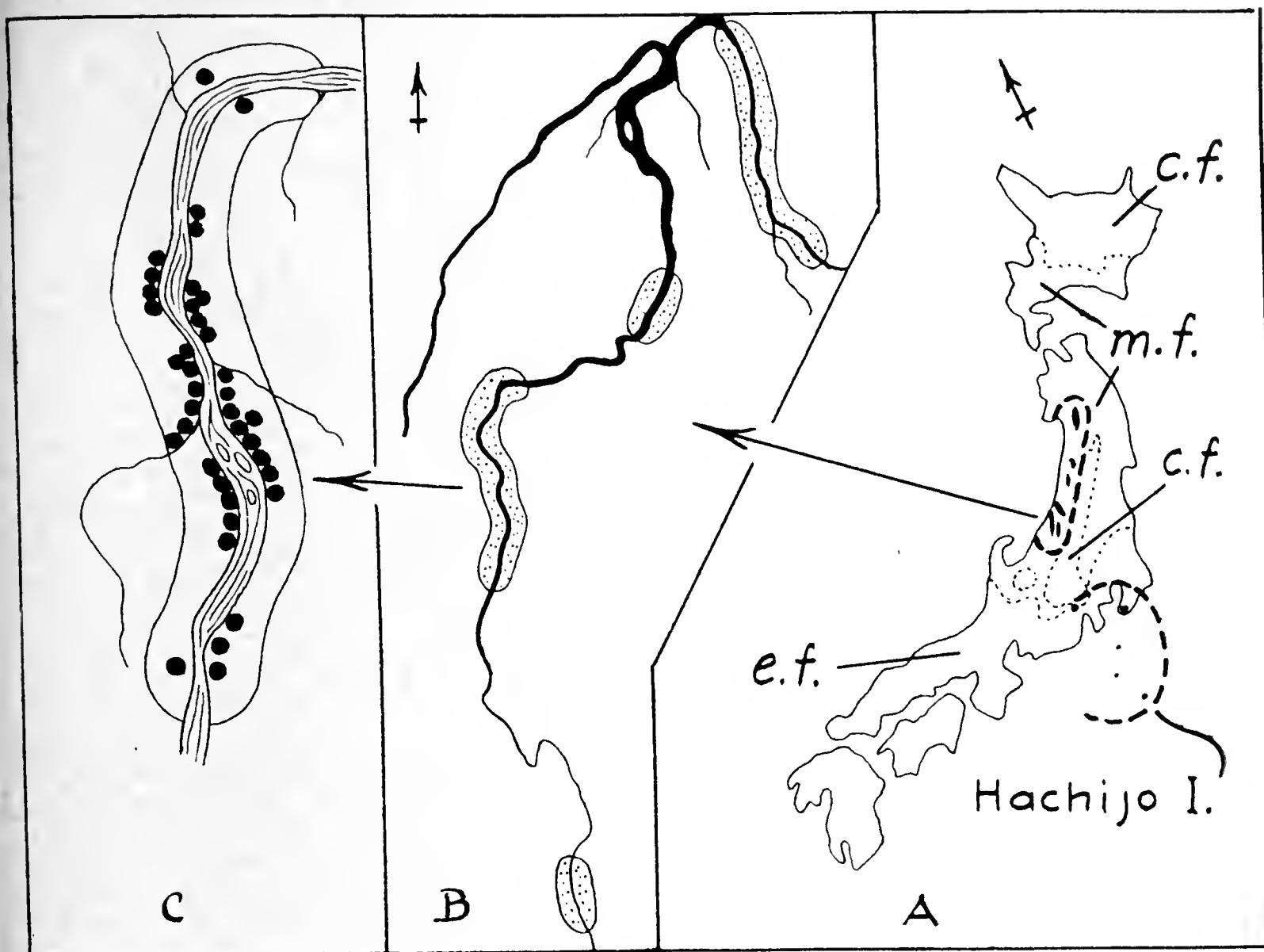


Fig. 2. Endemic areas and foci in Japan (after Kitashima & Miyagawa 1918, and Sasa 1954). A, showing two groups of endemic areas, of classical summer scrub typhus in northwest Honshu (to which *T. akamushi* is confined), and winter scrub typhus in southeast Honshu (*T. scutellaris*). Other endemic foci, all in the south, are omitted. *e.f.*, evergreen forests; *m.f.*, distinct mixed deciduous forest also found in eastern North America; *c.f.*, coniferous mountain forest. B, four endemic areas of classical type along the Shinano river, Niigata Prefecture, each corresponding to a river section subject to flood and having alluvial flats. C, some of the endemic areas showing highly infective foci or 'typhus-islands'.

#### LIFE-CYCLE

Both *T. akamushi* and *T. deliensis* may be described together. Eggs are laid in the soil and take 10–12 days to hatch at 30°C. The emergent six-legged larvae (chiggers) are about 220 x 150 $\mu$ , and orange or ochre-coloured. They may be seen to climb from the soil onto animals placed as bait or onto one's boots. They attach in closely packed clusters, usually in the ears of field rodents, feeding there on lysed dermal tissue for a period ranging in the laboratory, for both species, from 1 to perhaps 10 days with a mean feeding time of 2.7 days (Harrison 1957). Fully engorged larvae are about 550 x 400 $\mu$ . After detachment they return to the soil, spend some hours or even a day or so seeking suitable shelter, and then rapidly become akinetic and pass into a nymphocrystalis or nymphophane stage, from which an eight-legged nymph emerges after about a week. The nymph is delicate, and is carnivorous: in the laboratory it feeds on eggs of mosquitoes and collembolans. After a week or more of active feeding, the nymph passes into a second pupa-like stage (teleiophane), also lasting about a week, from which the adult emerges. The adult resembles the nymph but is larger and sexually mature. It feeds like the nymph on eggs and resting stages of other arthropods. In the laboratory egg-laying may start after the first 10 days of adult life, a healthy female fed daily laying 30–40 viable eggs a month. Egg-laying may continue for at least a year. A strain of *T. deliensis*, started by Mr. K. L. Cockings from a single female, has been kept in our laboratory through 17 generations.

The life-cycle, from egg to egg-laying, has been completed in the laboratory for *T. deliensis* in Malaya in under 40 days, and for *T. akamushi* in Japan in under 70 days (Sasa and Miura 1953). The duration of the life-cycle in nature cannot readily be discovered in equatorial climates, but there is evidence that it took about 10 weeks during the hot-wet season (June to August) in Imphal on the Indo-Burma border in 1945 (Audy and Harrison 1951). Life-cycles in relation to climate are discussed at the end of this paper.

## BEHAVIOUR OF LARVAE

Two most important aspects of behaviour of the larval chiggers are in their method of attachment and feeding, and their response to microclimate. They have favourite sites of attachment which vary somewhat with the species of host and also the locality (discussed by Audy 1956c), but the commonest site is the thin inner epithelium of the ears of rodents. They may take several hours to attach, tending to do so in closely packed clusters. One effect of this appears to be a tendency to leave the host in unison, presumably owing to the disturbance of neighbours by the act of detachment, and even to shorten the collective feeding-time (Harrison 1957b). Another effect may concern the ready transfer of rickettsiae if these remain in numbers where they are inoculated by infected chiggers: this is a point for investigation.

Responses to microclimate are very important: in brief, the larvae tend to disappear from the surface of the soil in the late morning after the dew has evaporated. There is a diurnal fluctuation in the availability of larvae associated with the daily changes in humidity, and fluctuations over longer periods associated with dry or wet spells of weather. Prolonged dry spells, or the onset of dry seasons, affect not only the larvae but also the adults which appear to lay fewer eggs or to cease egg-laying.

## RELATIONSHIPS BETWEEN THE VECTORS AND OTHER TROMBICULIDS

Gater (1932) in Malaya followed by Philip and Woodward (1946) in the Philippines and Philip (1947) and Tanaka (1954) in Japan have cast doubt on the separation of the two major vectors as distinct species on morphological grounds; and Philip and Kohls (1948) reduced *deliensis* to a variety of *akamushi* pending further studies. In Malaya, the two behave as distinct species and are also morphologically separable especially if the overlap in scutal size is negated by taking a scutal-coxal ratio (Traub and Audy 1954: 74); but we have clear evidence that *akamushi* at least, and possibly *deliensis*, are introduced species in Malaya (Audy 1956b), so that we may have here the familiar situation of distinct subspecies being brought together by introduction from elsewhere and behaving as species. My own opinion is that a reasonable working hypothesis is to suppose that *akamushi* and *deliensis* represent dominant forms of a Palearctic-Malaysian cline which has become greatly confused by the repeated scattering of these mites over considerable ranges by birds. For some purposes (e.g. Harrison and Audy 1951) the two forms may conveniently be lumped.

As shown in Fig. 3, *T. akamushi* in Malaya is practically confined to the field-rat, *R. argentiventer* Kloss, and birds such as quail in patches of open grassland, while *T. deliensis* is much more widespread on a greater variety of hosts and types of terrain although it is most numerous on *R. argentiventer* in grassy scrub or a rat occupying the same niche (e.g. the house-rat *R. r. diardii* in grass in Singapore). The two forms may occur together on the same host, a situation which also obtains in New Guinea and other places. A distinct form which had been confused with *T. deliensis* on a giant-rat in the forest fringe has now been described as *T. (L.) langati* Audy, and a similar confusing species is *T. (L.) bodensis* Gun. in Borneo. *T. deliensis* is widespread in Borneo, but recent collections there have found relatively little *T. akamushi* but large numbers of a distinct form near *T. obscura* Wom. In Manipur, India, *T. (L.) fulleri* Ew. is a species closely similar to *T. deliensis*. *T. (L.) russica* (Ouds.) of Europe and *T. (L.) myotis* Ew. of North America (which may be form *a* of *russica*—Fuller 1952) very closely resemble *T. akamushi* but occupy a different niche (on bats).

Altogether over 50 species may be placed in a well-knit *akamushi*-group. These, together with perhaps 20 more species, at present make up the subgenus *Leptotrombidium*.

The subgenus *Leptotrombidium* (larvae of which have branched sensillae) is in Malaya practically confined to the ground-living giant-rats in forest, except for *akamushi* and *deliensis* which occur in large numbers outside it, although *deliensis* occurs in small numbers on the giant-rat *R. mülleri* near the edge of the forest. Of the other trombiculids dominant in Malaysia and shown in Fig. 3, most *Euschöngastia* (except *Walchiella*) infest canopy-dwelling mammals, while *Gahrlepiea*, *Walchiella*, and intranasal *Traubacarus* infest ground-living mammals. Larvae of all these have swollen sensillae. These latter genera appear to have evolved in the tropical and perhaps subtropical forests, but *Lep-totrombidium* appears to be relatively best developed in higher altitudes and especially

higher latitudes, its cradle of evolution perhaps being palaearctic rather than oriental. For example, in the Himalayan mountains, the U.S.S.R., and Japan, there are about 5 species of *Leptotrombidium* to every species of *Euschöngastia* s.l., but in Malaya there are some 3 species of *Euschöngastia* s. l. to every species of *Leptotrombidium*.

SMALL-SCALE DISTRIBUTION AND RELATIONSHIP TO HOSTS

This has been discussed for *T. deliensis* and other chiggers by Audy (1947, 1949), Audy *et al.* (1953: 37), Audy and Harrison (1951: 388), Harrison and Audy (1951: 172), and Harrison (1956).

Fig. 3 shows some chigger populations in Malaya, based on our collections and on estimates of chigger feeding-times made by my colleague J. L. Harrison. The areas of the squares are proportional to the number of chiggers completing their feeds on an 'average' host in unit time. The population-densities of the hosts are not indicated, but the species of rats which are outside the forest (C to F) are usually much more populous. The hosts selected for this chart represent (A) a typical tree-living, and (B) a typical ground-living rodent in the forest; (C and D) the semi-arboreal wood-rat *Rattus r. jalorensis*, in two types of

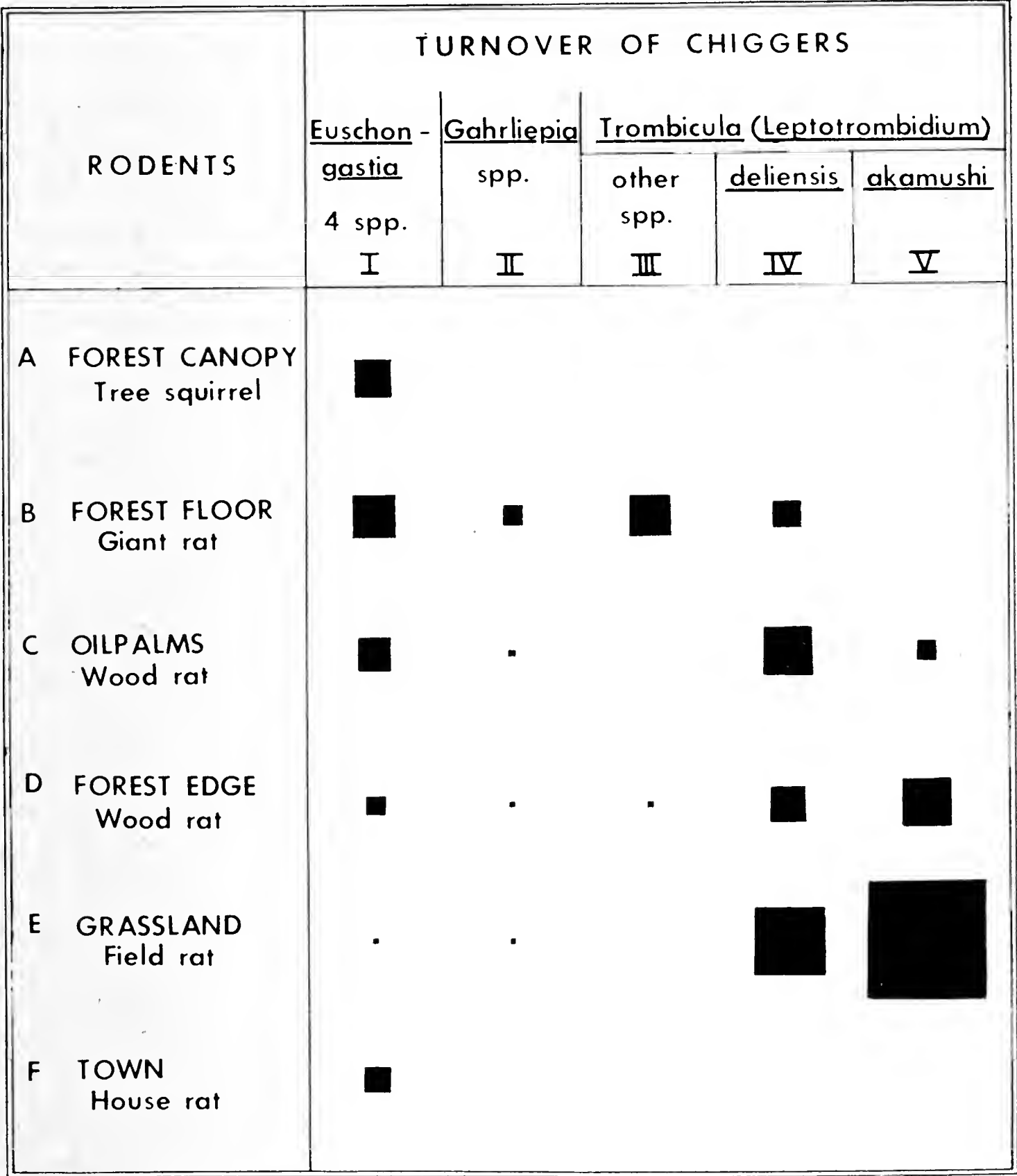


Fig. 3. Relationships of main groups of chiggers to hosts in Malaya. The four species of *Euschöngastia* s. l. occupy different niches: *E. (L.) audyi* in the canopy (A, and to some extent in D), *E. (W.) oudemansi* and *E. (W.) lacunosa* on the forest floor (B), and *E. (L.) indica* outside the forest (C,E,F) and to some extent in the forest-edge (D). A dominant species of *Leptotrombidium* in the forest is *T. (L.) langati*.

habitat, namely, an oil-palm estate, and the edge of grassland and forest which is this rat's natural habitat; then (E) the field-rat *R. argentiventer* which is an introduced rat practically confined to grassland, and finally (F) the introduced house-rat *R. r. diardii* in the town of Kuala Lumpur. The chiggers shown are dominant species or groups: (I) four dominant species of *Euschöngastia*; (II) several species of *Gahrlepiea* s.l., (III) species of *Leptotrombidium* closely related to the vectors; and finally (IV and V) the vectors *T. deliensis* and *T. akamushi*.

In brief, it appears (A) that chiggers are generally habitat-specific rather than host-specific although group-preferences (e.g. for reptiles, birds, mammals), definite host-preferences (e.g. one species of rat rather than another in the same general habitat), and occasionally apparent host-specificity are encountered; (B) that the building up of chigger populations is related to the frequency with which a host covers the same ground within the time which most of the chiggers take to feed (the mean feeding-time is below 3 days for the major vectors in Malaya, and higher, mostly 5 to 15 days or more, for other species; Harrison 1957b). For the latter reason, it is practicable to group hosts as (i) long-ranged, responsible for dispersal of chiggers (e.g. carnivores, birds, ungulates), (ii) short-ranged and occupying a suitable niche, responsible for building up local populations of chiggers, and (iii) short-ranged but occupying a niche which for some reason is unsuitable for the species of chigger (e.g. an arboreal rodent in the case of the major vectors).

In any particular locality it is usually possible to point to one or perhaps two species of animal as the major hosts of a particular chigger, each host occupying a particular niche. It is also possible to use the chiggers infesting a host to indicate its habitat and behaviour (Audy 1947, I.M.R. 1952: 50, Harrison 1957a). The most suitable hosts in the case of the major vectors are undoubtedly small mammals and certain ground-birds living in open grassy scrub.<sup>2</sup> Each individual tends to build up infestation in its home-range by repeatedly shedding engorged larvae in foci which are frequently revisited, and mark-recapture experiments in the field have clearly demonstrated the resulting self-infestation at least for *T. deliensis* in Malaya (Harrison 1956). The result is a patchy distribution of chiggers within the home-range of the hosts. The hosts themselves are not distributed at random; for example, a concentration of 5 to 10 individuals of one species per acre may adjoin an area from which that species is practically absent. Superimposed on this are features (e.g. soil moisture) which affect the chiggers rather than their hosts. The net result is the irregular distribution of chiggers in characteristic 'mite-islands'. Two overlapping mite-islands of *Helenicula kohlsi* (Ph. and W.) and *H. lanius* (Rad.) (as *mutabilis*, err. det.) were described by Audy (1947).

## ENDEMIC SCRUB TYPHUS TRANSMITTED BY THE MAJOR VECTORS

### RICKETTSIAL INFECTION IN MAJOR VECTORS

The causal agent, *Rickettsia tsutsugamushi* Ogata, has been shown to be transmitted vertically (transovarially) from adult to larvae in both the major vectors. Experiments with Dr. S. R. Savoor in Malaya (IMR 1950: 66, 1952: 54, Audy and Harrison 1951: 402) on a pure strain of *T. deliensis* suggested that transmission to following generations may not be very efficient: further studies are indicated. Evidence suggests that a chigger might harbour recoverable rickettsiae but be unable to transmit them, while the possibility of latent avirulent phases, as with the rickettsiae of Rocky Mountain spotted fever, cannot be denied although our experiments suggested that eggs and adults may harbour rickettsiae in a virulent phase. Though the evidence is sketchy, it appears that recoverable infection may be present in the field in from perhaps 1 in 10 to 1 in 1000 or more larvae; larvae capable of transmitting infection may form perhaps 5 per cent or even more of the total in one focus but extremely low rates of infection are common. The question of varying virulence has been discussed by Audy and Harrison (1951: 403) and Audy (1954): much experimental work requires to be done. It is clear that a non-random distribution of rickettsiae is superimposed on a non-random distribution of chiggers: the result which may be expected is that actually encountered in the field, namely the presence of many mite-islands only some of which may be 'typhus-islands'.

<sup>2</sup> Interesting facts which require explanation are, first, that *T. akamushi* appears to attack birds such as quail in much larger numbers than does *T. deliensis* in our collecting areas in Malaya; and secondly, that the small *Rattus exulans concolor* is often found in Malaya living in the same patch of grassland together with *R. argentiventer* but in these circumstances the latter always carries much larger numbers (e.g. x20) of *deliensis*, although other sub-species of *R. exulans* are the major hosts where they occur alone in scrub in the Pacific area.



### ENDEMIC AREAS AND ENDEMIC FOCI

The geographical distribution of scrub typhus is roughly that of the major vectors which have evidently reached oceans and deserts which practically bar further dispersal of the vectors by birds. This area of distribution covers over five million square miles of land. Within it, endemic scrub typhus is distributed patchily into endemic areas or regions, and within these areas infection occurs in isolated foci or 'typhus islands' which may be only a few yards across.

Climate, which is discussed briefly below, plays a major part in determining the distribution of scrub typhus. The drier or more inhospitable the climate, the more endemic foci become confined to streambanks, seepages, irrigated areas, or parts of peoples' gardens. This is decided partly by the preference of the mites for moisture, and to a lesser extent by the habitat-preferences of the hosts. In the barren Pescadores islands, which are swept by sea-winds, infected foci are restricted to small clumps of grass in gardens protected by coral walls within which live the rats. In Japan, *T. akamushi* is at the northern limit of its range and, according to Sasa (1954), it is there practically confined to alluvial banks on certain rivers in north-west Honshu (the classical areas of tsutsugamushi disease). This part of Japan has a distinctive climate and a peculiar type of forest which is also found around Maine, U.S.A. but nowhere else (Asa Gray 1873), so that some comparative studies of chigger-faunas should be profitable. In Australia, scrub typhus is practically confined to a small coastal strip of tropical Queensland which alone receives adequate rainfall, but it is likely that there are irrigated and otherwise suitable places for its maintenance further south. In the dry parts of India and central Burma, foci may be almost confined to tanks, seepages in protected hollows, or domestic waste land which is kept moist (e.g. Fort Dufferin in Mandalay and the gardens of the richer people in Jamshedpur).

The relationship to vegetation is extremely instructive, and has been partly discussed by Audy (1949); later work, yet unpublished, has thrown further light on the 'edge-effects', which are now familiar to the ecologist. The conversion, usually by man, of uniform tracts of vegetation into a mosaic of seral units has a profound effect on the animal life. New niches are created, and one of these (grassy open scrub in southeast Asia) has been exploited by field-rodents and has apparently given a great evolutionary impetus to the genus *Rattus*. Also, concentrations of certain animals and their attendant chiggers are encouraged at the edges ('fringe-habitats') between vegetation units, examples of such effects on *T. deliensis* being given by Audy (1947).

Endemic foci may persist for an indefinite number of years but they are not static. New foci are known to be created in suitable circumstances, and other foci may be suddenly destroyed or slowly become uninfected. These changes are generally related to land utilization, flooding, and changes in the plant-animal community structure with the regeneration of forest.

## JUNGLE TSUTSUGAMUSHI AND THE EVOLUTION OF ENDEMIC SCRUB TYPHUS

### OTHER TROMBICULIDS AS VECTORS OF RICKETTSIAE

Information is available about the following species of chiggers.

Species of *Leptotrombidium* in Japan: *Trombicula* (L.) *pallida* Nagayo et al. Attacks man; known to have caused an infection; rickettsiae recovered from larvae taken in the field; epidemiological evidence that it is the vector in some foci near Tokyo. The commonest and most widespread chigger in Japan, but apparently not a universal vector. *T.* (L.) *scutellaris* Nagayo et al. Strong epidemiological evidence that it is the sole vector of the mild 'winter scrub typhus' (Shichito fever) of south Honshu and islands below Yokohama. *T.* (L.) *palpalis* Nagayo et al., *T.* (L.) *intermedia* Nagayo et al., and *T.* (L.) *tosa* Sasa and Kaw. have been suspected on epidemiological grounds of transmission in specific parts of Japan. Information on all the above species has been reviewed by Sasa (1954).

Species of *Laurentella* in the Malaysian subregion: *Euschöngastia* (*Laurentella*) *audyi* (Wom.) is a dominant chigger on tree-living rodents in the forests of Malaya and Borneo. *Rickettsia tsutsugamushi* was recovered from this species by Traub et al. (1950). *Euschöngastia* (L.) *indica* (Hirst) is almost the only urban chigger in this whole region. Gispen (1950b) recovered *R. typhi* (*R. mooseri*), the agent of urban flea-typhus, from *E. indica*.

taken from house-rats in Java. Ecological and other information about species of *Laurentella* is summarized by Audy (1956a).

Species of *Gahrlepiea* s.l. in the Indian subregion: *G. (Schöngastiella) ligula* (Rad.) is widespread in north India and Burma and is often found on house-rats. Kalra (1947) reported recovery of *R. tsutsugamushi* from this species in India. He noted that it was impossible to exclude the chance inclusion of some *T. deliensis* in the pool of chiggers, but because larvae of *G. ligula* and *T. deliensis* are easily recognized by their different colours, his evidence in favour of infection in *ligula* is strong.

Species which cause scrub itch: *Eutrombicula wichmanni* (Ouds.) is the western Pacific counterpart of the common American *E. alfreddugèsi* (Ouds.). Under the mistaken name of *T. minor* it was suspected to be a major vector in Australia and New Guinea, but there is in fact no valid evidence in favour of this and positive evidence that it does not transmit. It is common in those parts of Queensland which are free from scrub typhus and absent from the endemic foci there. On Hachijo island, Japan, it is present together with the presumed vector *T. scutellaris* but attacks man in the summer while scrub typhus occurs in the winter when *T. scutellaris* is prevalent. Philip (1949) fed the closely related *E. splendens* (Ew.) on an infected rat and showed that although rickettsiae were actually ingested they did not appear in the next generation. *Schöngastia schüffneri* (Walch) (= *pusilla* (Wom.), synonym) another scrub-itch chigger, was reported by Walch and Keukenschrijver (1923) as causing an exceptional infection in a labourer in Sumatra. This was in a focus where scrub typhus was being actively transmitted by *T. deliensis*. People were being attacked by *S. schüffneri* mostly in the adjoining forest, and not in the endemic focus where cases occurred, so that *S. schüffneri* was obviously not transmitting infection significantly and the report needs confirmation. Both *Eutrombicula* and *Schöngastia* are essentially genera of reptile-chiggers (Audy 1952) a few species of which have a wide host-range including man. All the others noted above are essentially mammal-chiggers, which do not cause scrub-itch.

It is therefore clear that chiggers in at least two and probably three different genera of mammal-chiggers carry *R. tsutsugamushi* in nature and are almost certainly capable of transmitting infection to the animals on which they feed. *Euschöngastia audyi*, *E. indica*, and *G. ligula* are not known to attack man, nor is there at present epidemiological evidence against them; but it must be noted that apart from the major vectors of scrub typhus and chiggers causing scrub-itch very little is known of the readiness with which other chiggers will attack man. I believe that many species will do so if given the opportunity, but it is obvious that the major vectors and the scrub-itch chiggers alone have sufficient opportunity to be important to us. *Leptotrombidium*, *Eutrombicula*, and *Schöngastia* differ from the other groups discussed above in having species which occur locally in very large numbers on the ground where man is exposed to their bites. Those species of *Leptotrombidium* which infest field-living rodents occur in this manner and they include the important vectors of scrub typhus. Some species of *Eutrombicula* and *Schöngastia* cause scrub-itch and evidence is against their involvement in scrub typhus, although it must be admitted that in foci where enzootic infection is maintained by efficient vectors, casual infections of other species of chiggers might occur.

We may therefore conveniently group trombiculids in the area of distribution of scrub typhus as follows:

1. Reptile-chiggers which bite man, cause scrub-itch, but do not transmit rickettsiae significantly (*Eutrombicula*, *Schöngastia*).
2. Mammal-chiggers which may be unable to transmit *R. tsutsugamushi* or may be able to do so with varying degrees of efficiency, in which case they are associated with enzootic infection. The latter may be divided into:—
  - A. Major vectors of universal importance to man (*T. akamushi*, *T. deliensis*).
  - B. Major vectors of local importance (apparently confined to certain palaearctic species of *Leptotrombidium* such as *pallida*, *scutellaris*, etc.).
  - C. Efficient vectors which may attack man but do not occur in sufficient numbers or in suitable habitats to infect man significantly. These may theoretically be responsible for sporadic cases or rare anomalous outbreaks. This is a hypothetical group to which *E. audyi* may belong.
  - D. Vectors which do not attack man but may be responsible for enzootic infection.

## EVOLUTION OF ENDEMIC SCRUB TYPHUS

This subject has recently been reviewed by Audy (1954). The natural vegetation over nearly the whole of the area of distribution of scrub typhus is forest and it appears that *R. tsutsugamushi* is normally present as a silent enzootic ('jungle tsutsugamushi') in at least the Malaysian forests, transmitted by trombiculids among both tree- and ground-living animals. Deforestation on a vast scale by man has created a new niche—grassy waste land—which has been exploited particularly by field-rats of the genus *Rattus* in the south and voles and other field-rats in the north. The *akamushi-deliensis* complex has evolved in the new favourable conditions and these mites have been dispersed widely, particularly by birds. Although the forest is very rich in species of animals, individuals of each species are relatively dispersed. In sharp contrast, the patchwork of mixed vegetation which follows deforestation is much poorer in species of animals, individuals of which however tend to occur in relatively dense colonies. In these conditions, the rickettsia native to the forests (either *R. tsutsugamushi* or a parent form) has been greatly developed and at the same time brought into contact with man. Most endemic scrub typhus may be fairly described as man-made and a consequence of faulty land-utilization.

## DISTRIBUTION OF SCRUB TYPHUS IN SPACE AND TIME

### DISTRIBUTION OF SCRUB TYPHUS SUMMARIZED

We are now able to answer two of the questions posed at the beginning of this paper. The vectors are distributed patchily in mite-islands related to colonies of suitable hosts. Only a relatively small proportion of these mite-islands are infected and these are recognized as endemic foci. Endemic foci are grouped into endemic areas by features of geography and climate. They are extensive and often confluent in moist tropical conditions and become more localized and separated in dry or otherwise rigorous climates. The major vectors of universal importance are two sister species, *Trombicula akamushi* and *T. deliensis*, which have been widely dispersed but have reached formidable geographical barriers to further spread. Endemic scrub-typhus has evolved from a silent infection (jungle tsutsugamushi) in forest chiggers in special circumstances created in southeast Asia by deforestation, which has led to a tremendous development of field-rodents in a niche which greatly favours the major vectors. It is reasonable to suppose that rickettsiae related to the parent form of *R. tsutsugamushi* exist in trombiculids in other continents but there has been no biological event comparable to that in southeast Asia to evoke an endemic counterpart to scrub typhus. The corresponding rickettsiae in ticks, however, have in every continent been taken up by particular tick-host associations with the evolution of local forms of tick-typhus. I would regard rickettsialpox as a special local development from a tick-borne parent form. The organism of Q fever spreads so easily in the absence of ticks in which it apparently had its evolutionary origin that it is no longer possible to guess at its cradle of development. Flea-typhus and louse-typhus have been spread round the world by man from centres of origin probably located in central Asia and eastern Europe.

### INFLUENCE OF CLIMATE

Fig. 4 shows four different types of seasonal incidence of the vectors of scrub typhus. The first is an example of a distinct 'typhus-season' in a monsoon climate. The onset of the rains is followed by a sudden high peak incidence of chiggers duly followed by a sudden increase in absolute numbers presumably due to the arrival of the next generation. Evidence was obtained (Audy 1947) that *T. deliensis* may pass through at least three life-cycles a year in the region of the Indo-Burma border, the cycles taking over two months during the hot-wet season and over three months in cool weather. Even more cycles may be passed on foci such as seepages and other places which remain moist through the dry season; such foci incidentally offer a special risk of infection to man. Except in such scattered foci, the life-cycle is interrupted and egg-laying apparently ceases for some months through the dry season.

The conditions shown in an equatorial climate such as that of Malaya are very different. There are no sharp peaks in populations, with all the biological implications such fluctuations carry. Transmission takes place throughout the year, with minor fluctuations due to drier or wetter periods. There is no seasonal interruption, the temperature remains at about 80–90°F, and it should be possible for five or more life-cycles to be passed in a year.

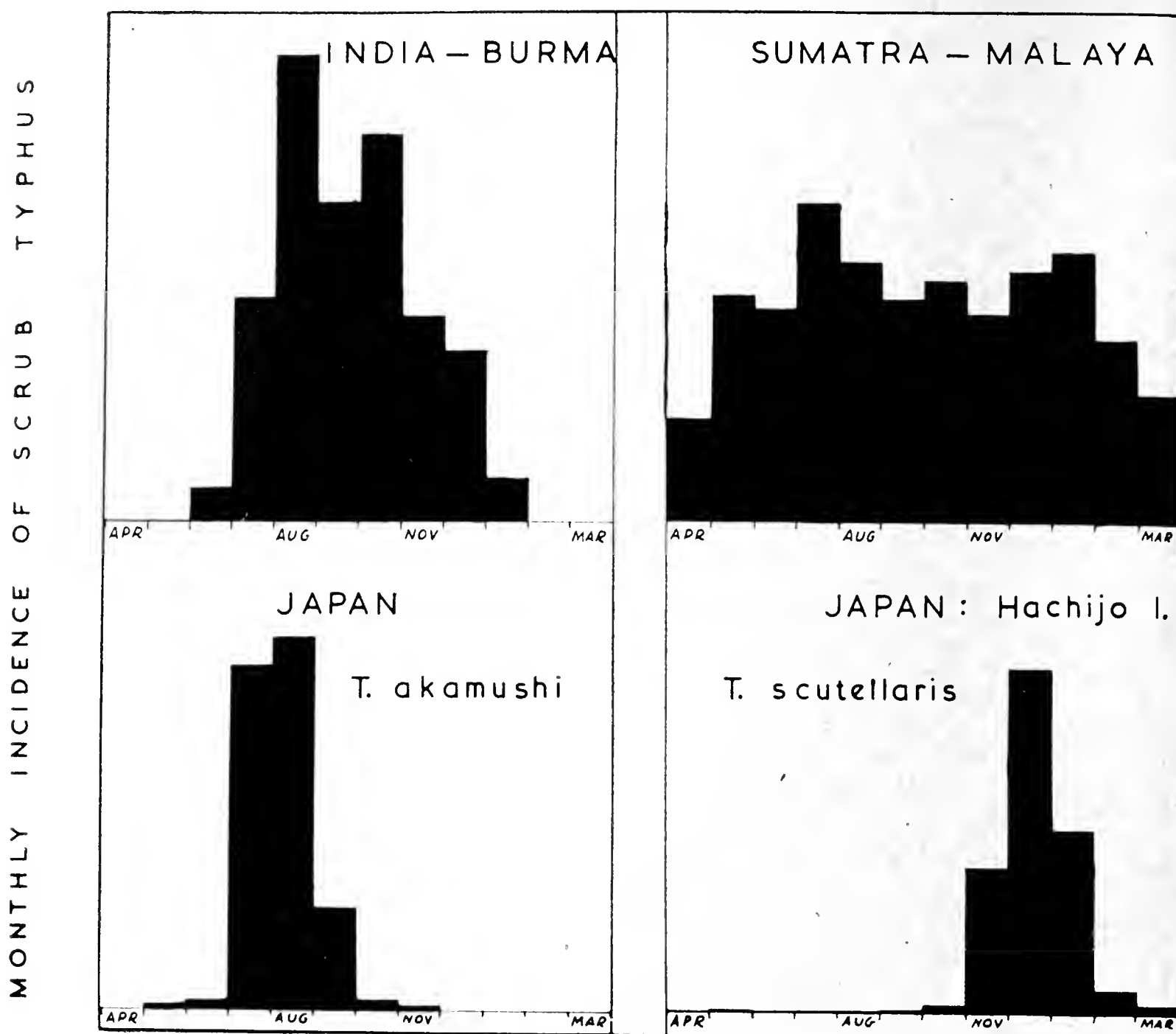


Fig. 4. Four types of seasonal incidence. Data for India-Burma from Audy & Harrison 1951; for Sumatra-Malaya, unpublished; for Japan, from Sasa (1954).

The situation with *T. akamushi* in Japan resembles that of the monsoon climate and there is a short sharp typhus-season. The *akamushi* here has a single annual cycle, interrupted for some six months by the winter. Larvae hatched in spring reach the adult stage in autumn and lay eggs the following spring (Kawamura and Ikeda 1936).

Finally, again in Japan, near the northward limit of distribution of scrub-typhus, several species of *Leptotrombidium* related to *T. akamushi* apparently bite man and transmit infection in certain localities. These species also usually have a single annual life-cycle but with different seasonal incidence according to the species. The example in Fig. 4 is of autumn-winter transmission by *T. (L.) scutellaris*. The presence of some twenty species of *Leptotrombidium* in Japan, the dominant species showing different seasonal incidences, is extremely interesting. A possible course of evolution leading to the separation of such species is discussed by Audy (1954b: 133).

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# A Helminth Replaces the Usual Arthropod as Vector of a Rickettsialike Disease<sup>1</sup>

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## ABSTRACT

Salmon poisoning disease of canines along the north-central Pacific Coast of the United States is caused by a rickettsialike pathogen that is carried by endoparasitic trematodes. Infection has been demonstrated in immature flukes found in certain indigenous snails, and in a high proportion of infested salmonid fish fed to dogs. The infection is presumed but not proved to pass in the eggs to new fluke generations. Probable organisms have been seen in sections of adult flukes in situ and suspensions of these are infectious. Since there is high mortality in untreated canine animals and no other suitable, susceptible host has been found, the reservoir mechanism remains a mystery. An intriguing hypothesis considers the possibility that polyembryonic proliferation of immature flukes in snails could provide a unique adaptation for starting of "new lines of infection" analagous to the rodent-tick mechanism in Rocky Mountain spotted fever.

## INTRODUCTION

We are so accustomed to associating arthropods with the transmission of certain animal disease agents caused by intracellular rickettsialike parasites that it comes as a startling discovery to find a helminth taking over the vector role in such a disease.

The agent of the so-called "salmon poisoning disease" of canines on the Pacific Coast of the USA is carried by very small, parasitic trematodes. The name originated with early settlers who noted high mortality among dogs that ate salmon or trout trimmings and carcasses along streams in western Washington and Oregon, and in northwestern California. A hypothesis for the scarcity of coyotes in those areas envisioned this infection as the cause. Fatality of over 90 percent in dogs was reported and until recently treatment was very ineffectual. It was also noted that the few dogs that did recover were solidly immune to reinfection and could eat raw fish with impunity. Occasional fox farmers in the enzootic areas had unfortunate experiences in the unknowing use of raw fish trimmings in their fox food.

The history and documentation of the early investigations was reviewed by the writer last year (Philip, 1955). As early as 1911, Pernot noticed the minute encysted flukes in local fish, but he thought they were amoebae. Donham (1925) correctly identified these as encysted flukes and associated them with the disease in dogs. The trematodes were soon after named *Nanophyetus salmincola* Chapin. Neither human cases of the disease nor fluke parasitism in man have been reported in the endemic area. The writer ate portions of raw, infested Oregon trout (aliquots of which caused fatal infection when fed to a dog) but no ill effects resulted, though a few fluke eggs appeared in the human stools ten days after ingesting the fish. A human trematode parasite in East Siberia, *N. schikobowi*, is considered by Witenberg (1932) to be a synonym of *N. salmincola*.

The redial and cercarial stages of the fluke develop only in pleurocerid snails, *Goniobasis silicula*, which inhabit the clear, running West Coast streams inhabited also by the salmon and trout hosts. Evidence is strong that the disease is limited to the restricted geographic distribution of this snail. During a field trip this summer (June, 1956) the writer obtained verbal reports from veterinarians of infected dogs as far north as Forks on the Olympic Peninsula, Washington, as far south as Eureka, California, and as far inland as the upper tributaries of the Rogue River above Grants Pass, Oregon. Over 200 canine cases annually were reported by veterinarians in two communities on both sides of the California-Oregon boundary, an area where every unleashed dog is considered sooner or later to have the opportunity of eating fish refuse along the streams. *Goniobasis* snails were taken on casual search between Centralia, Washington, and Crescent City, northern California, and between sea level in smaller streams just above high tide mark to remote Rogue River tributaries in the mountains at nearly 3000 feet altitude.

<sup>1</sup> From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases.

<sup>2</sup> With the technical assistance of E. Lyndahl Hughes. Dr. W. J. Hadlow performed the histopathological studies.

Fortunate it is that neither the snail nor the disease are known farther north into Alaska where salmon are a seasonal, staple diet among sled dogs.

It was formerly believed by dog owners in enzootic areas that fish were not only susceptible to the disease but were the direct cause of "poisoning" dogs that ate the carcasses. However, we failed to produce recognizable disease in trout fingerlings which were injected with infectious dog node suspensions, and penned in a local, Bitterroot stream. Suspensions of tissues from these fish prepared 15 and 3 days after injection failed to infect dogs, which confirms the circumstantial evidence that trout (in this case fluke free) are not themselves affected.

### THE DISEASE IN DOGS

The disease in the dog has often been described but may be briefly characterized as an acute systemic infection usually initiated in 2 to 8 days after exposure by high fever, complete loss of appetite, marked mental depression, rapid decline in weight, and often vomition and bloody diarrhea which appears to result from the infection rather than fluke parasitism *per se*.

We have studied the disease in over 100 dogs infected mostly by fish feeding, or injection of infectious materials. The high natural fatality rate is substantiated experimentally. An astonishingly high proportion of cyst-carrying, Oregon trout fingerlings has caused infection when fed to our experimental dogs. Occasional dogs that recover spontaneously or following treatment have resisted subsequent challenge with infectious material. This fact is used by many owners of valuable dogs in the enzootic areas, who deliberately "salmon" their dogs to cause infection, and then have them treated to induce lasting immunity. No vaccine has been developed.

Others (Simms *et al.*, 1932; Cordy and Gorham, 1950) have shown, and we have confirmed the observations that the sulfonamides are useful in treatment intravenously, and that antibiotics particularly aureomycin and terramycin (as little as one 250 mg. capsule by mouth for a desperately ill 15-pound beagle) are dramatically effective.

The chief lesions found at necropsy include enlarged, visceral lymph nodes and variably inflamed intestinal mucosa, including the colon beyond the usual area of heavy fluke infestation in fish-fed animals. Suspensions of lymph nodes are infectious by injection or application to scarified skin of other dogs, but not by feeding or instillation into their eyes; node tissues remain infectious for long periods in the frozen state.

### THE ETIOLOGIC AGENT

It was not until the studies of Cordy and Gorham (1950) that intracellular organisms were discovered in lymph node preparations, and they correctly concluded that this agent belonged in the order Rickettsiales. Organisms have been found in many tissues of our infected dogs, and we (Philip, Hadlow, and Hughes, 1953) named them *Neorickettsia helminthoeca*, the first rickettsialike agent found to be transmitted by a vector other than an arthropod. This requires broadening of the definition of the family Rickettsiaceae from "arthropod-borne" to "invertebrate-borne."

The agent does not survive indefinite passage in the usual laboratory animals, white mice and guinea pigs, nor will it grow in embryonated chicken eggs, contrary to most typhuslike pathogens of Rickettsiaceae.

Suspensions of washed, adult flukes from fatally infected dogs are infectious by injection, and we have infected dogs in two instances by the injection of suspensions of snail livers which were determined by prior dissection to contain the stub-tailed, simple-armed cercariae of *Nanophyetus*. A Seitz-pad filtrate of a suspension of adult flukes was not infectious though an unfiltered, simultaneous aliquot produced the disease. Probable organisms have been seen in sections of flukes *in situ* in intestinal crypts of our infected dogs by Dr. W. J. Hadlow.

### THE DISEASE CYCLE AND NATURAL RESERVOIR

It becomes obvious that the agent is adapted to a fantastic vector cycle in nature. Presumably infected eggs of the fluke contaminate natural waters from suitable host feces. The larval flukes or miracidia penetrate *Goniobasis* snails, perhaps only singly, where they multiply polyembryonically to produce hundreds of eventual cercariae. These are liberated



in mucous strings from which, in contacting salmonid fish, the cercariae penetrate fish tissues, often through natural openings, and encyst in various organs and muscles. The flukes mature in the intestines of various fish-eating vertebrates but only Canidae (dogs, coyotes, foxes) develop salmon poisoning. Bears (Farrell and Gorham, correspondence) and raccoons which eat fish and are indigenous in the enzootic area have not developed experimental infection. The flukes have not been reported in fish-eating, coastal birds.

The natural reservoir mechanism is still a puzzle since the canine fatality rate appears too high to account for the high incidence of infection in cyst-carrying fish. To test the possible intervention of some ectoparasite of vertebrates, we tried transmission through three species of ticks. *Dermacentor andersoni* failed to become infected in any stage. One mechanical transmission occurred by interrupted feeding of adult *Haemaphysalis leachi* between an infected and a normal dog. Three other transmissions occurred by bites of nymphs and adults, but not larvae, of *Rhipicephalus sanguineus* fed on an infected donor in the previous adult generation. Part of these second generation ticks also produced the disease when injected into dogs but the positive results were too sporadic to postulate this as a subsidiary explanation for natural maintenance. Hypothetical progenitors of the agent might have been acarids in the geologic past.

A more likely explanation of the reservoir mechanism in the absence of other host evidence, appears to the writer to involve the polyembryonic proliferation of the fluke itself. Since only single or at most a few larvae (miracidia) of some other species of flukes (see Philip, 1955) are known to attack host snails to initiate polyembryony, then it may be postulated that the high proportion of fish that carry infected cysts of *N. salmincola*, is probably accounted for by multiplication of the disease agent during polyembryonic multiplication of immature flukes in the snails. This would be an unique mechanism of natural maintenance of a rickettsialike pathogen, but in a way analogous to the starting of new lines of Rocky Mountain spotted fever rickettsiae in immature ticks feeding simultaneously on a susceptible rodent bitten by an infected tick.

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# The Occurrence of Some Insect-Borne Diseases in Suriname in Relation to the Distribution of their Vectors

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## ABSTRACT

Suriname (Dutch Guiana) may be divided into three zones, viz.: (a) the young coastal area; (b) a belt with sandy savannas, alternating with swamps and forests; (c) the somewhat hilly tropical rain forest, intersected by rivers.

### MALARIA

The most important malarial area is the tropical rain forest. Here falciparum malaria is hyperendemic. Though anophelines abound in the coastal area most parts are practically free of malaria; other parts however are heavily infected; there vivax and malariae infections prevail and falciparum malaria is scarce. The spleen indices in children in the bushnegro villages in the interior are very high. In the savanna belt, however, the indices vary from 6.6 (Para villages) to 80 along the Wayambo, and 100 (Langamankondre).

Falciparum malaria in Suriname is closely connected with the prevalence of Anopheles darlingi Root. It is obvious that one should associate the great differences of the spleen rates with the distribution of A. darlingi. Its permanent breeding places lie mainly along the large rivers south of the savanna belt. During the great rainy season it penetrates deep into the savanna belt along the rivers, and the creeks. In this way invasions of darlingi cause epidemics of falciparum malaria. The weather conditions govern the extension of the breeding places, explaining severe epidemics in regions which normally are malaria free.

### LEISHMANIASIS

Leishmaniasis is confined to the rain forest. In spite of the numerous captures of insects with human bait in the other areas no Phlebotomes were observed. Five species of man-biting Phlebotomes, P. intermedius, P. squamiventris, P. arthuri, P. guyanensis, and P. paraensis were captured in the endemic leishmaniasis area. P. intermedius is known as a vector of leishmaniasis, and it was captured in a village with many cases.

### BANCROFTI WUCHERERIASIS

Wuchereriosis is an important disease in the densely populated areas. Examination of 51,000 persons in Paramaribo showed that 17% were carriers of microfilariae. The vector, Culex pipiens fatigans (=quinquefasciatus) has numerous breeding places. In the rural areas the vector is also abundant but only a few cases of wuchereriosis occur; its failure to spread into the rural area may be attributed to (a) the limited flight range of its vector; (b) the greater distance between the houses; (c) most of the inhabitants being of asiatic origin and having arrived only one or two generations ago.

### OZZARDI FILARIASIS

Contrary to the wuchereriosis the ozzardi filariasis is confined to the original population, the American Indians. In the upper Suriname district Lampe observed microfilariae of ozzardi in 46.6% of the males, 42% of the females, and 19.5% of the children, whereas none of the 200 bushnegroes were carriers. In a heavily infested American Indian village a number of Culicoides guttatus were captured. In two specimens filarial larvae were observed in the proboscis, showing that probably C. guttatus is a transmitter of ozzardi filariasis. In Suriname the American Indians live isolated from the other ethnic groups. This fact and the limited flight range of C. guttatus explain why this parasite does not occur in other ethnic groups. The distribution of the vector is much wider than the endemic ozzardi filariasis area.





# Rôle des Insectes comme Hôtes intermédiaires dans les Cycles des Trématodes digénétiques

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## RÉSUMÉ

Cette étude passe en revue diverses particularités de la biologie des Trématodes parasites d'Insectes: spécificité, mode de pénétration, influence de la métamorphose sur le cycle du parasite, progénèse, réactions de l'Insecte parasite. Un certain nombre de cycles où interviennent des Insectes sont donnés en exemples.

On a signalé bien souvent la présence de formes larvaires de Trématodes (Métacercaires) enkystées chez des Insectes à divers stades: larves, nymphes et imagos. Ces parasites n'ont pas toujours pu être rattachés de façon certaine à une espèce de Trématode adulte; cependant, dans beaucoup de cas il a été possible d'élucider le cycle et de préciser le comportement de l'Insecte et de son parasite.

Le but du présent travail n'est pas d'établir un catalogue complet des Trématodes trouvés chez les Insectes, mais de présenter quelques remarques sur la biologie de ces parasites et de résumer quelques cycles.

Des représentants de toutes les classes de Vertébrés peuvent être contaminés par des Insectes porteurs de métacercaires.

## SPÉCIFICITÉ PARASITAIRE

Très souvent les Insectes se comportent comme des hôtes occasionnels, dépourvus de spécificité: certaines métacercaires peuvent être hébergées aussi bien par une larve d'Insecte aquatique quelconque que par un Crustacé, un Oligochète ou un Turbellarié: c'est le cas, p. ex., de *Pleurogenes medians* (Olsson). Il est évident que le cycle présente alors des possibilités plus étendues suivant les hasards qui amènent les cercaires en présence de l'hôte.

Dans d'autre cas, le choix est plus étroitement limité: les *Prosthogonimus* s'enkystent chez des Odonates; *Dicrocoelium dendriticum* (Rudolphi) chez des Fourmis.

Les Trématodes d'Insectes sont, naturellement plus fréquents chez les espèces qui passent une partie ou la totalité de leur vie dans le milieu aquatique; mais ils existent aussi chez des espèces terrestres: Chrysomélides, Formicides.

## MODE DE PÉNÉTRATION

On peut distinguer plusieurs modes de pénétration des cercaires infestantes: 1°) Pénétration active à travers l'exosquelette. Le passage de la cercaire est alors contrôlé par des tropismes qui peuvent être modifiés dans certaines conditions expérimentales (voir J. Rioux et P. Quézel 1948; E. Brumpt 1944). *Xiphidiocercaria polyxena* Brumpt pénètre chez des larves de Diptères tuées par la chaleur aussi bien que chez des larves vivantes. L'action mécanique de la ventouse et du stylet est, en général, complétée par une action chimique due aux produits élaborés par les glandes de pénétration.

2°) Pénétration passive: la cercaire est ici entraînée par le courant d'eau dans la chambre branchiale chez une nymphe d'Odonate. C'est le cas de *Plagitura salamandra* F. J. Hull (1928) chez divers *Sympetrum* et *Leucorrhinia*; c'est également le cas de *Prosthogonimus macrorchis* Macy (1934) chez *Leucorrhinia*.

3°) Contamination par voie digestive. C'est probablement par ce processus que s'infestent les larves de Chrysomélides hôtes de *Brachylecithum* (J. F. Denton 1945) et les Fourmis hôtes de *Dicrocoelium* (W. H. Krull et C. R. Mapes 1952).

## INFLUENCE DE LA MÉTAMORPHOSE SUR LE CYCLE DU PARASITE

La métamorphose de l'Insecte entraîne pour le Trématode un changement de biotope qui a des conséquences importantes pour sa destinée. Ce changement peut être, suivant le cas, favorable ou défavorable pour le cycle: beaucoup de Trématodes de Chiroptères (*Lecithodendriidae*) sont parasites de larves aquatiques de Diptères et de Trichoptères.

Après la métamorphose, les kystes persistent et se trouvent alors dans des conditions favorables pour parvenir chez l'hôte définitif.

Le cas inverse existe aussi: mes recherches dans le Sud-Est de la France (1956) ont montré que certains Odonates (*Sympetrum striolatum* Charp.) au stade adulte renferment des métacercas de *Prosotocus* dans la proportion de 22%: il s'agit là de parasites égarés qui n'ont que peu de chances de parvenir chez leur hôte normal (*Rana*): la contamination est en général assurée par les larves ou les nymphes.

#### PROGÉNÈSE

La progénèse est fréquente chez les métacercas parasites d'Insectes: de nombreux cas ont été cités depuis les observations de A. Villot (1870) (voir à ce sujet A. Buttner 1951-52). Le développement des œufs chez les larves enkystées pourrait avoir la signification d'un cycle abrégé (expériences de W. W. Crawford (1940) sur *Dytiscus*).

#### RÉACTIONS DE L'INSECTE

La présence des métacercas enkystées est, en général, supportée par les Insectes sans dommage apparent. Mes recherches sur *Sympetrum striolatum* Charp. ont montré que les sujets parasités ont un comportement normal et volent parfaitement malgré la présence de 30 à 35 kystes de *Prosotocus*.

A. Ch. Hollande (1920) a étudié les réactions histologiques d'un *Dytiscus* infesté par 65 métacercas. W. Neuhaus (1940) a signalé la formation de pigments mélaniques dans la paroi du kyste (voir également A. Buttner loc. cit.). Cette réaction est fréquente; je l'ai observée régulièrement dans les xénokystes de *Prosotocus* développés chez *Sympetrum striolatum* Charp.

#### EXEMPLES DE CYCLES OÙ INTERVIENNENT DES INSECTES

##### EPHÉMÉROPTÈRES

Ces parasites évoluent chez des Poissons et des Batraciens. C'est le cas de *Crepidostomum farionis* O. F. Müll. (*Allocreadiidae*) qui est parasite à l'état adulte de la Truite. Le cycle a été étudié par F. J. Brown (1927). Le premier hôte est un Lamellibranche (*Pisidium*, *Sphaerium*). Les cercas s'enkystent dans le corps adipeux et les muscles d'*Ephemera danica* Müll., particulièrement dans la région ventrale, entre la seconde et la cinquième branchie. La contamination du poisson peut avoir lieu aussi bien par ingestion des larves que par celle des imagos. Les kystes peuvent être nombreux (jusqu'à 36 chez un seul sujet). De la même façon, *Crepidostomum cooperi* S. H. Hopkins (1931) est transmis à divers poissons par *Hexagenia limbata* Guérin.

*Allocreadium isoporum* (Looss 1894) est un parasite répandu chez de nombreux poissons d'eau douce: Brochet, Carpe, Barbeau, Tanche, Brème etc. Le cycle ressemble au précédent: cercas développées chez *Sphaerium*, métacercas chez *Ephemera vulgata* L. (et aussi chez des Trichoptères).

*Dolichosaccus rastellus* (Olsson 1876) (*Plagiorchiidae*) est très commun dans le tube digestif de *Rana esculenta*. Premier hôte: *Limnaea ovata* Drap. deuxième: *Ephemera vulgata* L. ou *Chloeon dipteron* L. (et aussi Trichoptères).

##### PLÉCOPTÈRES

Ces Insectes contaminent des Batraciens (*Opisthioglyphe*), des Oiseaux (*Plagiorchis*) et des Chiroptères (*Lecithodendrium*).

##### ODONATES

Les Odonates hébergent un très grand nombre de métacercas appartenant aux familles des *Plagiorchiidae*, *Lecithodendriidae*, *Gorgoderidae*, *Halipegidae*. En voici quelques exemples: *Alloglossidium corti* (Lamert) est un parasite de Poisson (*Ameiurus*). La contamination est assurée dans la nature par *Leucorrhinia intacta* et, dans les conditions expérimentales, par *Tetragoneuria cyanosura*, *T. spinigera*, *Gomphus spicatus*. Les cercas, entraînées dans la chambre respiratoire, traversent la paroi du rectum et s'enkystent dans les muscles et le tissu adipeux de l'abdomen (W. W. Crawford 1937).

*Pneumonoeces variegatus* (Rudolphi) et *P. similis* (Looss), parasites des poumons de la Grenouille *R. esculenta* sont transmis par des Zygoptères (*Agrion virgo* L.); *P. sibericus* Bychowski par des Anisoptères (*Sympetrum frequens*).

*Plagitura salamandra* Holl 1928 est parasite à l'état adulte du Batracien *Triturus viridescens*; les métacercaires sont enkystées chez des nymphes de *Sympetrum* et de *Leucorrhinia* (et aussi chez des Hémiptères Notonectides. (H. M. Owen 1946) *Pleurgoenes liberum* (Seno 1907) est parasite chez diverses Grenouilles du Japon; la métacercaire correspondante a été trouvée enkystée chez *Crocothemis servilia* et *Orthetrum albistylum* (Okabe 1937).

D'autres représentants de la même famille (*Lecithodendriidae*), très répandus chez les Grenouilles d'Europe: *Pleurogenes medians* (Olsson) et *Prosotocus confusus* (Looss), passent aussi par des Odonates (*Sympetrum* en particulier).

Les *Gorgodera* (Fam. *Gorgoderidae*), adultes dans la vessie des Grenouilles, ont leurs métacercaires chez diverses espèces d'Agrion, *Epithea*, *Enallagma* etc.

Dans la famille des *Halipegidae*, *Halipegus ovocaudatus* (Vulpian) est localisé à l'état adulte dans la trompe d'Eustache de *Rana esculenta*; son cycle comporte comme premier hôte diverses espèces de Planorbes et comme second hôte *Agrion virgo* L. (D. F. Sinitsin 1905). La métacercaire d'*Halipegus occidialis* Stafford a été trouvée par H. W. Krull chez *Libellula incesta* Hagen dans le Maryland.

Le genre *Prosthogonimus* Lühe (1899) (Fam. *Plagiorchiidae*) groupe une série de Distomes qui, à l'état adulte, sont parasites dans la bourse de Fabricius de divers Oiseaux domestiques et sauvages (voir, en particulier, à ce sujet le travail de R. Ph. Dollufs 1948). Ces Trématodes ont un certain intérêt vétérinaire (Prosthogonimose des volailles). Les métacercaires sont enkystées chez des larves, nymphes et imagos d'Odonates: *Leucorrhinia*, *Tetragoneuria*, *Gomphus*, *Epicordulia*, *Epithea*, *Anax*, *Libellula* etc. (Recherches de L. Szidat 1928-31, R. W. Macy 1934-39, S. Ono 1928 etc.). Le kyste a une double paroi très épaisse, la couche interne étant homogène et la couche externe striée radialement. L'infestation est particulièrement abondante dans la musculature ventrale de la région postérieure de l'abdomen. On a constaté que la prosthogonimose se manifeste souvent à la suite de migrations amenant en grand nombre les Insectes porteurs de métacercaires. Certains Oiseaux sauvages peuvent jouer le rôle de réservoirs d'Helminthes et sont ainsi à l'origine de l'infestation des Oiseaux domestiques. Mes recherches en Provence (1953) ont montré que la Pie est souvent parasitée par des *Prosthogonimus* et peut ainsi contribuer à disséminer la prosthogonimose.

#### MÉGALOPTÈRES

La larve de *Sialis lutaria* L. est très fréquemment infestée par des métacercaires de *Pleurogenes medians* (Olsson). A. Buttner (1950-51) qui en a fait une étude détaillée signale un taux de parasitisme atteignant 40% dans certains gîtes. (adulte chez la Grenouille).

M. Lühe (1909) mentionne chez cette même larve la métacercaire de *Plagiorchis maculosus* (Rud.) (adulte chez *Hirundo* et *Apus*).

#### TRICHOPTÈRES

Les Trichoptères hébergent souvent des métacercaires; les adultes correspondants se trouvent chez des Poissons, des Batraciens, des Oiseaux et surtout des Chiroptères.

*Allocreadium isoporum* (Looss), déjà cité à propos des Ephémères, peut aussi être transmis aux Cyprinidés par *Anabolia nervosa* Leach. et *Chaetopteryx villosa* F. Le Plagiorchide *Dolichosaccus rastellus* (Olsson) peut être transmis aux Grenouilles par divers *Limnophilus* et *Anabolia*. *Plagiorchis maculosus* (Rud.) peut être communiqué aux Hirondelles et Martinets par *Drusus trifidus* M. Lachl.

Parmi les parasites de Chiroptères, on peut citer comme exemple le cas de *Prosthodendrium chilostomum* (Mehlis) Braun (1900). L'adulte est répandu chez de nombreux hôtes: *Plecotus auritus* L., *Myotis mystacina* (Kuhl.), *Nyctalus noctula* (Schreb.), *Eptesicus serotinus* (Schreb.). La cercaire correspondante pénètre chez la larve de *Phryganea grandis* L. et demeure sans s'enkyster dans l'abdomen (hémocèle et corps adipeux). A la métamorphose, il y a migration dans les muscles thoraciques où se produit l'enkystement.

R. A. Knight et J. Pratt (1955) ont élucidé récemment deux autres cycles: *Allasogonoporus vespertilionis* Macy et *Acanthatrium oregonense* Macy. Les adultes se trouvent tous deux chez *Myotis lucifugus* (Le Comte); les métacercaires des deux espèces sont transmises par des *Limnophilus* sp.

## COLÉOPTÈRES

On a publié une série d'observations concernant la présence de métacercaires chez des Coléoptères. Dans les cas les mieux connus, il s'agit de parasites évoluant chez des Grenouilles et chez des Oiseaux.

W. F. Harper (1928) a signalé, chez la larve de *Dytiscus marginalis* L. des kystes correspondant à une cercaire qu'il a appelée *Cercaria* XI. Ch. Joyeux (1930) a obtenu expérimentalement la pénétration de la cercaire de *Pleurogenes medians* (Olsson) chez cette même larve.

La métacercare d'*Haplometra cylindracea* (Zeder) a été trouvée par von Linstow (1890) enkystée chez *Ilybius fuliginosus* F. (larve et imago).

On doit à J. F. Denton (1945) la découverte intéressante du rôle de certains Chrysomélides (*Gastroidea cyanea* Schiff.) dans le cycle de *Brachylecithum americanum* Denton. Les larves, exposées à la pénétration des cercaires sur des feuilles de *Rumex* ont été trouvées infestées par 1 à 6 métacercaires au bout d'un temps variant de 1 à 5 jours. Le ver adulte est parasite des voies biliaires de Corvidés et Ictéridés Nord-Américains.

## LÉPIDOPTÈRES

La métacercare de *Plagiorchis fuji* Ogata (1942) a été décrite par cet auteur chez *Stenopyche griseipennis*.

## DIPTÈRES

Les Diptères interviennent en particulier dans les cycles de Trématodes de Grenouilles et de Chiroptères. Une série de travaux ont été consacrés aux métacercaires de Culicidés. Alessandrini (1909) a signalé chez *Anopheles maculipennis* Meig. une métacercare attribuée à *Lecithodendrium ascidia* v. Bened. (adulte chez *Vesperugo pipistrellus*); d'autres observations sont dues à C. Joyeux (1918) chez *Culex hortensis*, à T. W. Jones (1950), à S. R. Christophers (1952).

M. A. Azim (1936) a étudié en Egypte la métacercare de *Lecithodendrium pyramidum* Looss chez *Anopheles pharaonis*. Les kystes passent sans changement chez la nymphe et l'imago; l'adulte est probablement l'hôte de *Vespertilio euryale*.

*Lecithodendrium lagna* (Brandes), parasite de nombreux Chiroptères, a été signalé à l'état de métacercaires chez *Chironomus plumosus* L., *Anopheles maculipennis* Meig. et d'autres insectes.

Les métacercaires ne sont pas rares chez divers Chironomides infestant des Grenouilles. *Opisthioglyphe ranae* Fröhlich 1907) s'enkyste expérimentalement chez des larves de *Chironomus*.

D'après van Thiel (1954) qui en a fait une étude détaillée, les *Anopheles maculipennis* sont parasités à Leyde dans la proportion de 5% par des métacercaires de *Pneumonoecus variegatus* (Rudolphi). L'évolution se poursuit dans le poumon de *Rana esculenta*.

## HYMÉNOPTÈRES

La découverte la plus remarquable de ces dernières années est certainement celle du rôle des Fourmis dans le cycle des Dicrocoeliidés. W. H. Krull et C. R. Mapes (1952-53) ont en effet montré que *Formica fusca* L. est le second hôte intermédiaire de *Dicrocoelium dendriticum* (Rud.). Ce cycle avait déjà donné lieu à beaucoup de discussions; il est maintenant élucidé. Les cercaires, issues de divers Mollusques pulmonés terrestres, sont éliminées par le pneumostome, incorporées dans des boules de mucus (Schleimballen). Les fourmis recueillent ces amas et les transportent dans leurs nids où elles se contaminent en grand nombre. Dans la nature, W. H. Krull et C. R. Mapes ont trouvé dans certains gîtes 35% de fourmis parasitées. Les métacercaires sont rassemblées particulièrement dans le gastre; les kystes mesurent 0,325 à 0,465 mm. Des contaminations expérimentales de divers Mammifères (Agneaux, Lapins et Cobayes) ont permis d'obtenir le ver adulte. Les travaux de P. K. Svadjian (1954) en Arménie et ceux de H. Vogel et J. Falcao (1954) en Allemagne ont confirmé cette découverte qui éclaire l'étiologie de la distomatose du mouton, maladie si répandue et si importante.

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# La Distribution de l'Espèce *A. elutus* sur le Territoire de la République populaire roumaine et son Importance comme Vecteur du Paludisme<sup>1,2</sup>

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## RÉSUMÉ

La République Populaire Roumaine présente des facteurs climatiques et orohydrographiques qui favorisent le développement d'un anophélisme représenté par les espèces: *A. claviger* (*bifurcatus*), *A. plumbeus* (*nigripes*), *A. hyrcanus* var. *pseudopictus*, *A. sacharovi* (*elutus*), ainsi que par le *A. m. typicus*, *A. m. messeae* et *A. m. atroparvus*, qui font partie du groupe *maculipennis*. *Anopheles sacharovi* (*elutus*) et les variétés du groupe *maculipennis* sont les vecteurs du paludisme. Dans la formule anophélienne d'une localité ou d'une zone déterminée, on rencontre rarement les vecteurs du paludisme en population pure, le plus souvent associés en des proportions variées. La prépondérance de l'une ou de l'autre de ces espèces ainsi que la grande différence de leurs relations avec l'homme imprime des caractères différents à l'endémie palustre de notre pays. C'est ainsi que la présence de l'espèce *A. sacharovi* sur le littoral de la Mer Noire a entretenu l'endémie pendant la période 1933-1948, à un grade élevé, culminant dans les années 1944-1946 par une puissante vague hyperépidémique (Tulcea). On présente des données relatives à la répartition de l'espèce *A. sacharovi*, à l'évolution annuelle et saisonnière, au grade d'anthropophilie et à l'index d'infection spontanée, en comparaison avec les observations enregistrées dans la même zone pour les autres facteurs. On montre les modifications survenues dans la faune anophélienne respective sous l'action des insecticides remanents hydrocarbures-chlorés, utilisés pour combattre le paludisme, ainsi que la diminution considérable de l'endémie palustre dans cette zone dans laquelle continue à persister l'espèce *A. sacharovi*.

Le territoire de la République Populaire Roumaine, situé entre 43°-48° latitude nord et 20°-30° longitude est, sous l'influence d'un climat continental excessif, présente des conditions favorables au développement de l'anophélisme sur près de 30% de la surface totale du pays. La variation des facteurs qui déterminent le caractère de la faune anophélienne permet l'existence des espèces suivantes identifiées jusqu'à présent: *A. maculipennis* avec les variétés *A. m. typicus*, *A. m. messeae* et *A. m. atroparvus*, *A. sacharovi* (*elutus*), *A. hyrcanus*, var. *pseudopictus* (*M. sinensis*), *A. claviger* (*bifurcatus*) et *A. plumbeus* (*nigripes*). (Leon, 1910, Zotta, 1932). Parmi ceux-ci, vecteur du paludisme sont les *A. sacharovi* (*elutus*) et les variétés du groupe *A. maculipennis*. Les espèces et les variétés vecteurs sont rencontrées rarement sous forme de populations pures ou presque pures, le plus souvent elles sont associées dans des proportions variées. (Lupaşcu 1945, Martini et Zotta 1934, Missiroli, et al., 1933, Zotta 1938, Zotta, et al., 1939).

Quoique l'image anophélienne d'une localité ou d'une région n'a de valeur que pour l'époque où l'on a fait les captures, les variations annuelles et saisonnières pouvant mener quelquefois à des inversions totales, nous pouvons cependant esquisser des zones où le caractère physicochimique presque constant des surfaces larvigères a favorisé pendant de longues années la prédominance des mêmes vecteurs.

Ainsi, *A. m. typicus*, qui est moins rencontré dans la plaine, prédomine dans les zones de collines sous-carpathiques et d'érosion. Sur le plateau moldave, il dépasse 75%, pourcentage inférieur à celui rencontré dans les vallées moyennes et supérieures des affluents du Danube. Cet anophèle, qui est un vecteur du paludisme en Moldavie, n'est plus transmetteur dans les conditions de température plus basse des zones d'altitude.

*A. m. messeae* est caractéristique pour les zones de plaine à eaux stagnantes permanentes ou temporaires. On le rencontre en pourcentages élevés et quelquefois même à l'état de population pure dans la plaine inondable du Danube, la vallée inférieure du Pruth, le

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Delta et le lac de Brăila. Étant un bon vecteur du paludisme dans notre pays, il a pu entretenir un degré élevé d'endémicité dans la zone des rizières et dans la vallée non divisée du Danube, de Măcin à Tulcea.

*A. m. atroparvus*, identifié en pourcentages variables sur le littoral marin, pénètre profondément à l'intérieur du pays dans les zones à eaux soumâtres. Le seul anophèle semihibernant dans les conditions de notre pays, *A. m. atroparvus*, est un des plus importants vecteurs.

L'espèce *Anopheles sacharovi* (*elutus*), caractéristique pour le climat circumméditerranéen et de l'Asie Mineure et connue comme le vecteur le plus dangereux du paludisme en Europe Méridionale, dans les pays circumméditerranéens et le proche Orient, (Barber et Rice 1935, Kligler et Mer 1930, Kligler 1932, Kligler et Mer 1932, Martini et Zotta 1934, Missiroli, *et al.*, 1933, Rice et Barber 1931) se trouble dans notre pays sur une zone limitée de l'est de la Dobroudja, qui représente le point le plus nordique de la diffusion de cette espèce. *A. sacharovi* (*elutus*) est plus strictement adapté aux zones de marais salants que le *A. m. atroparvus*. Les conditions optimales de salinité pour les stades larvaires de cette espèce semblent se trouver entre 5–10‰. (Hackett et Missiroli, 1935, Missiroli, *et al.*, 1933). La capacité d'adaptation des larves peut pourtant varier entre des limites très larges; elles se maintiennent dans les eaux douces et aussi dans des eaux à salinité variant de 0,5‰, 1‰, 2‰, jusqu'à 15‰–20‰. (Zotta, 1943). Ses particularités biologiques et ses manifestations oecologiques lui font maintenir, chez nous aussi, le caractère de redoutable vecteur du paludisme. Dans cette aire de limite nordique de distribution de l'espèce *A. sacharovi*, apparaissent pourtant certaines modifications dans sa biologie, que nous considérons utile de faire connaître dans ce qui suit.

Dans notre pays, les observations commencées en 1933, date à laquelle la présence de cette espèce a été signalée sur le littoral sud de la Dobroudja par Martini et Zotta (1934) ont été continuées jusqu'à présent. Pour l'intervalle 1933–1942, la direction scientifique revient en totalité au Professeur G. Zotta. (Cantacuzino et Lupaşcu 1944, Duport 1940, Duport et Atanasiu 1949, Săndulescu 1944, Trifon 1944, Zotta 1938, Zotta, *et al.*, 1939, Zotta 1943, Zotta, *et al.*, 1943, Zotta, *et al.*, 1944). Les recherches concernant l'espèce *A. sacharovi* dans notre pays, commencées en premier lieu au sud de la Dobroudja, zone dans laquelle on a identifié en 1933 les premiers exemplaires, ont été étendues progressivement vers le nord.

Dans cette étude on a poursuivi: l'établissement de l'image anophélienne des localités de cette zone par l'identification des ovipositions des femelles capturées dans les étables et les habitations; l'enregistrement de la fréquence comparative de l'espèce *A. sacharovi* dans les habitations et dans les écuries; l'enregistrement de l'indice d'infection spontanée des anophèles capturés; la variation de la faune anophélienne et l'évolution de l'endémie palustre en rapport avec les mesures instituées pour la combattre.

#### RÉPARTITION DE L'ESPÈCE *A. sacharovi* (*elutus*): VARIATIONS ANNUELLES ET SAISONNIÈRES

Toute cette étude est basée sur l'examen pour l'identification de 65,603 anophèles et 92,734 exemplaires disséqués provenant de 69 collectivités afin d'établir l'indice oocystique et sporozoïtique. L'espèce *A. sacharovi* (*elutus*) a été identifiée dans la majorité des localités (43) soit pendant plusieurs années pour les unes, soit une seule année pour les autres.

En commençant avec les années 1948, 1949, lorsque dans le cadre de l'action anti-paludique on a fait, dans les habitations et les écuries de la majorité de ces localités, des pulvérisations antiimago avec des insecticides de type rémanent, DDT ou HCH, l'espèce *A. sacharovi* est identifiée en des pourcentages de plus en plus réduits. Cette situation était due, d'une part, à la baisse considérable des densités anophéliennes, conséquence des pulvérisations, et, d'autre part, à la diminution de l'indice de survivance des anophèles capturés qui, étant transportés dans les stations de lutte antipaludique pour compléter les observations, arrivaient en contact avec des traces d'insecticide pénétré involontairement et contre les indications dans l'enceinte des laboratoires, et ne pouvaient plus évoluer, ni pondre.

Les études systématiques entreprises en 1955, avec la suspension des désanophélisations antiimago dans quelques localités où, auparavant, l'*A. sacharovi* se trouvait en de grands pourcentages, et les possibilités créées par l'utilisation de la méthode de détermination des anophèles (femelles) morts récemment ou moribonds par "l'identification des ovipositions



extraites par dissections" (M. Săndulescu, 1957) ont eu pour résultat la détermination d'un plus grand nombre d'anophèles et l'identification de l'espèce *A. sacharovi* dans le cours de l'année 1955 dans 19 des 38 localités investiguées dans son aire de répartition. (Fig. 1).

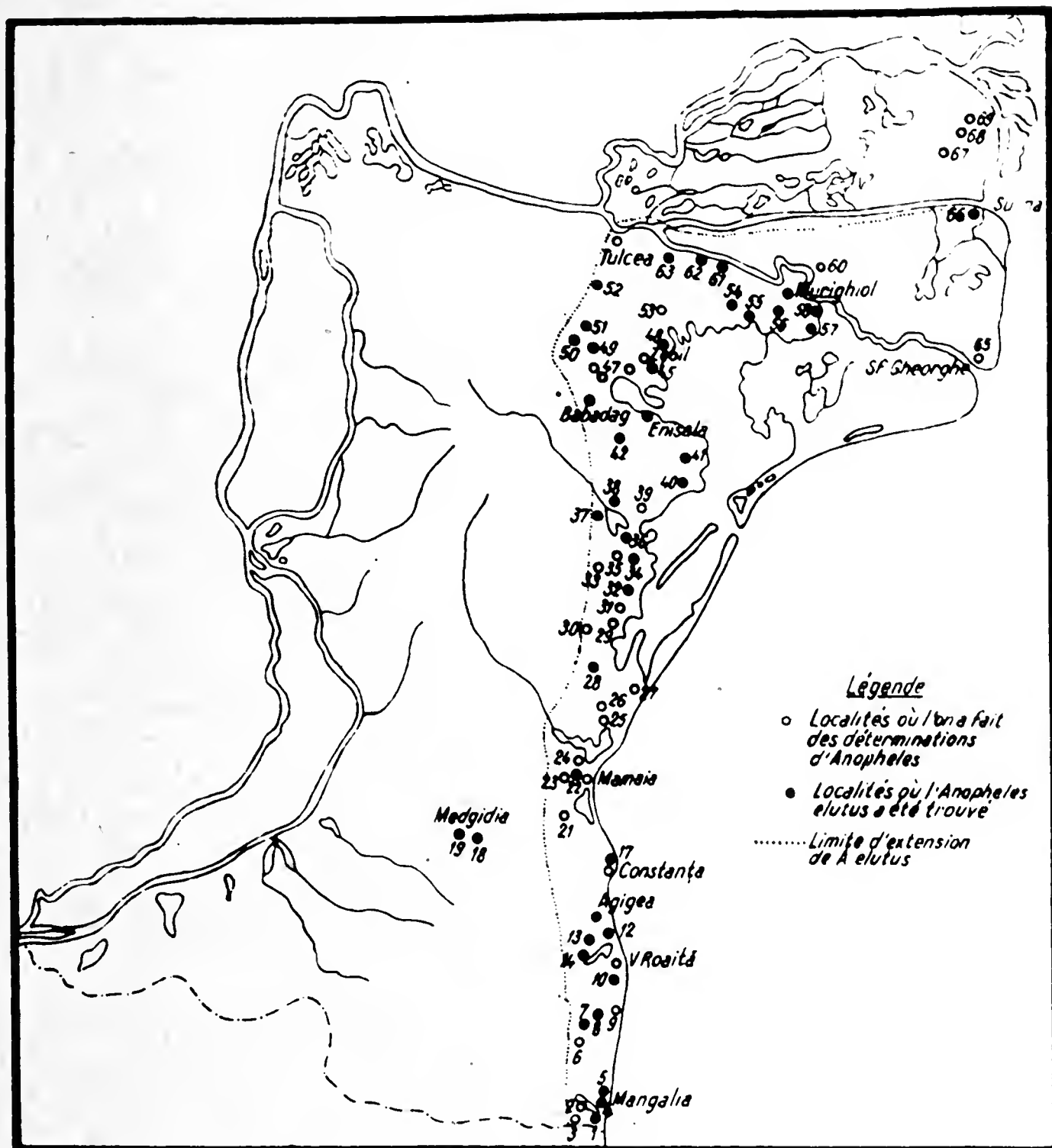


Fig. 1. La zone de distribution de l'espèce *A. sacharovi* (*elutus*) en Roumanie.

Toute l'aire de répartition de cette espèce se trouve à l'est de la Dobroudja, sur une bande de littoral marin de 12 à 15 km. de largeur qui, à partir de la localité Corbul de Sus s'éloigne de la mer du côté de laquelle s'interpose le complexe des grands lacs (Razelm, Golovița, Zmeica, Sinoe): elle avance vers le nord jusqu'à Tulcea, port danubien situé à approximativement 60 km. à l'ouest de la mer Noire, et jusqu'à Sulina, dans le Delta, située dans la zone du littoral marin. Les limites d'expansion sud et nord sont représentées par les localités "Două Mai" et Sulina, qui se trouvent à une distance d'environ 160 km. l'une de l'autre. Un seul point de pénétration à l'intérieur, isolé comme une île, est représenté par les localités Medjudia et Castelu, situées dans la vallée du Cara-Su, à 35-40 km. à l'ouest du littoral.

Parmi les causes de modifications survenues dans la formule anophélienne des localités de cette zone, outre la variation des facteurs climatiques, on doit compter aussi les modifications déterminées par l'intervention de l'homme: assainissement, création de nouvelles surfaces larvigères, application des insecticides rémanents, etc.

De l'examen des dates concernant l'évolution anophélienne de la localité Agigea, localité qui—avec deux années seulement d'interruption— a été étudiée avec continuité dans l'intervalle 1933-1955, ainsi que par l'analyse des modifications qui se sont produites dans les conditions de milieu de la même période, il résulte que parmi les causes qui, dans l'intervalle 1935-1948 ont déterminé la modification des proportions dans la formule ano-

phélienne, a contribué aussi l'exécution des travaux d'assainissement de la principale source anophélienne (le lac Agigea). Ces travaux, commencés en 1936 et non terminés, se dégradent déjà au cours de l'année 1938. Ensuite, nous assistons, en 1939, à une diminution procen-  
tuelle des variétés de *A. m. messeae* de 63.97%–41.74%, *A. m. atroparvus* de 29%–11.15% et une augmentation pour l'espèce *A. sacharovi* de 3.75%–43.54% (Fig. 2).

Dans les variations enregistrées de l'anophélisme de la localité Agigea, entre les années 1937–1947 inclusivement, se reflètent les modifications naturelles des conditions de milieu. Dans cet intervalle, à partir de 1940, dans la formule anophélienne prédomine l'*A. m. atroparvus*, variant entre 50.74% et 98.76%, tandis que l'espèce *A. sacharovi* qui, entre 1939–1940 enregistre 43.54%–40.95%, décroît beaucoup dans les années suivantes, sans dépasser 8.6% pour être totalement absente pendant l'année 1944. Les données enregistrées pour la période 1948–1954, période de pulvérisation antiimago avec des insecticides rémanents, montrent, en commençant notamment par l'année 1949, une diminution progressive du nombre des déterminations par ponte, et, en ce qui concerne l'*A. sacharovi*, il n'a plus été identifié pendant les années 1953, 1954.

La suspension expérimentale des pulvérisations dans la localité Agigea, pour la première fois en 1955, et l'association de la méthode d'identification des "ovipositions extraites par dissection" permettent l'identification pendant cette même année pour la même localité, d'un plus grand nombre d'exemplaires—925, parmi lesquels 64.75% sont représentés par l'*A. sacharovi*.

L'activité de l'espèce *A. sacharovi* au cours d'une année revêt, dans notre pays, les aspects suivants: les femelles hibernantes commencent généralement leur activité hématophage, l'activité gonotrophique et le vol d'abri en abri, plus tard (au mois d'avril) que les variétés du groupe *A. maculipennis* (au mois de mars). Par exception, pendant les années à printemps chauds et précoces, on obtient des ovipositions appartenant à cette espèce aussi au mois de mars. Pendant les mois d'avril, mai, juin, on obtient un pourcentage réduit d'ovipositions de l'espèce *A. sacharovi*, pour enregistrer une augmentation brusque au courant du mois de juillet. Dans l'intervalle juillet-septembre, dans les conditions naturelles, on obtient les plus nombreuses ovipositions d'*A. sacharovi*, et même la prédominance de cette espèce dans certaines localités. A la fin du mois de septembre et au commencement du mois d'octobre, commence la baisse de l'activité gonotrophique avec la réduction brusque des ovipositions, plus tôt que pour le groupe *A. maculipennis*.

Dans notre pays, l'espèce *A. sacharovi* hiberne complètement pendant les mois d'hiver, les femelles s'abritant tant dans les écuries que dans les habitations. Les déterminations effectuées au cours de l'hiver (dans des conditions d'élevage) démontrent que cette espèce est présente dans les maisons aussi que dans les étables. La présence dans les étables en nombre comparativement plus grand en cette période de l'espèce *A. sacharovi* n'indique pas nécessairement la préférence pour ce type d'abri en hiver, mais plutôt qu'il offre plus de possibilités de retraite et de persistance que les habitations.

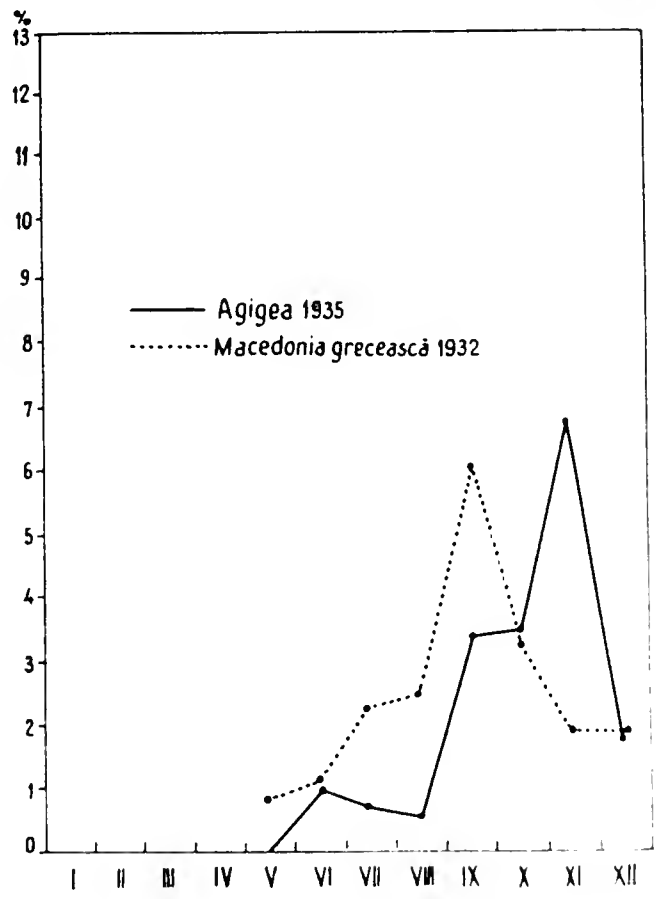
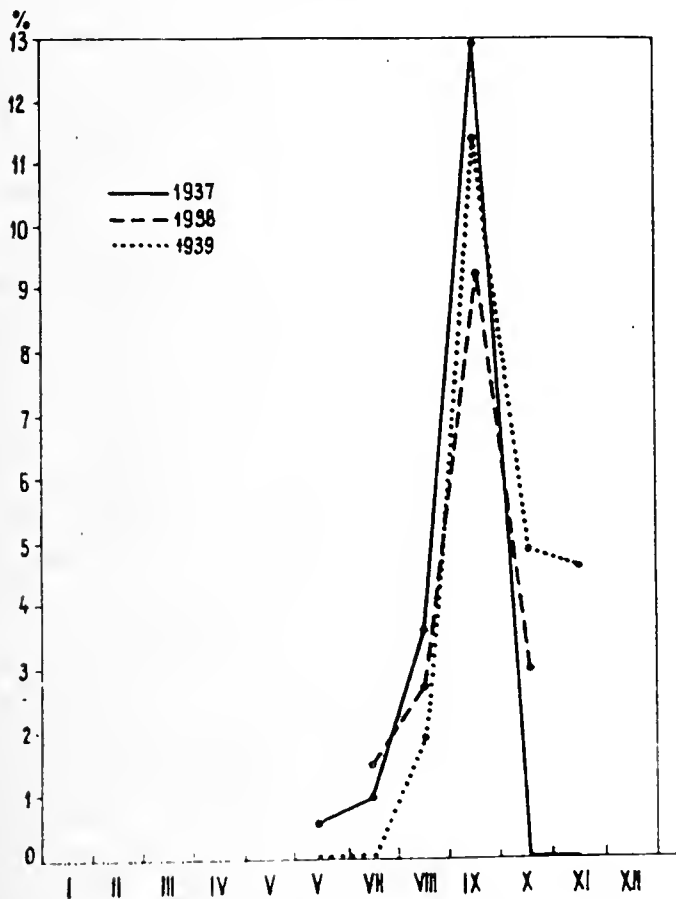
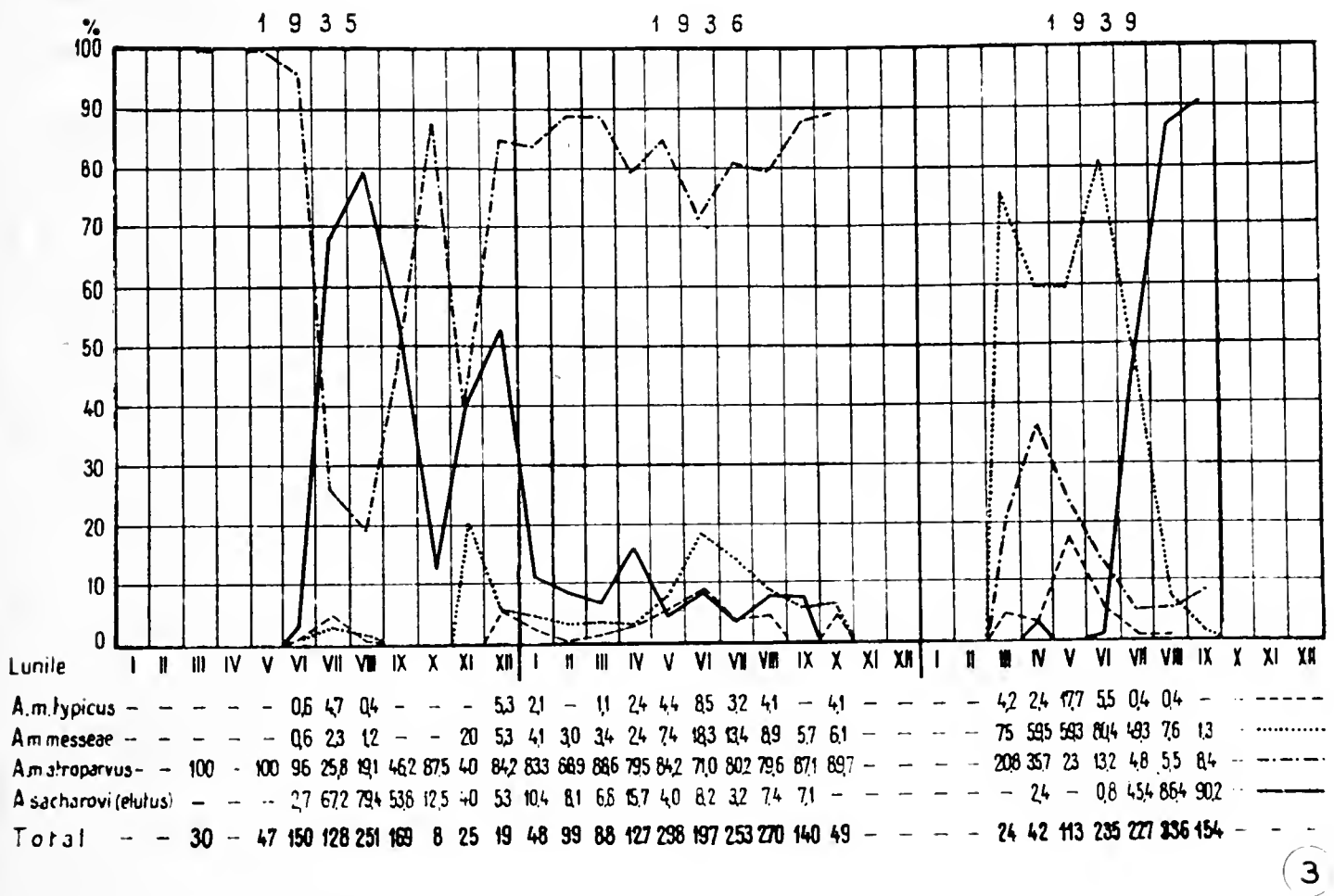
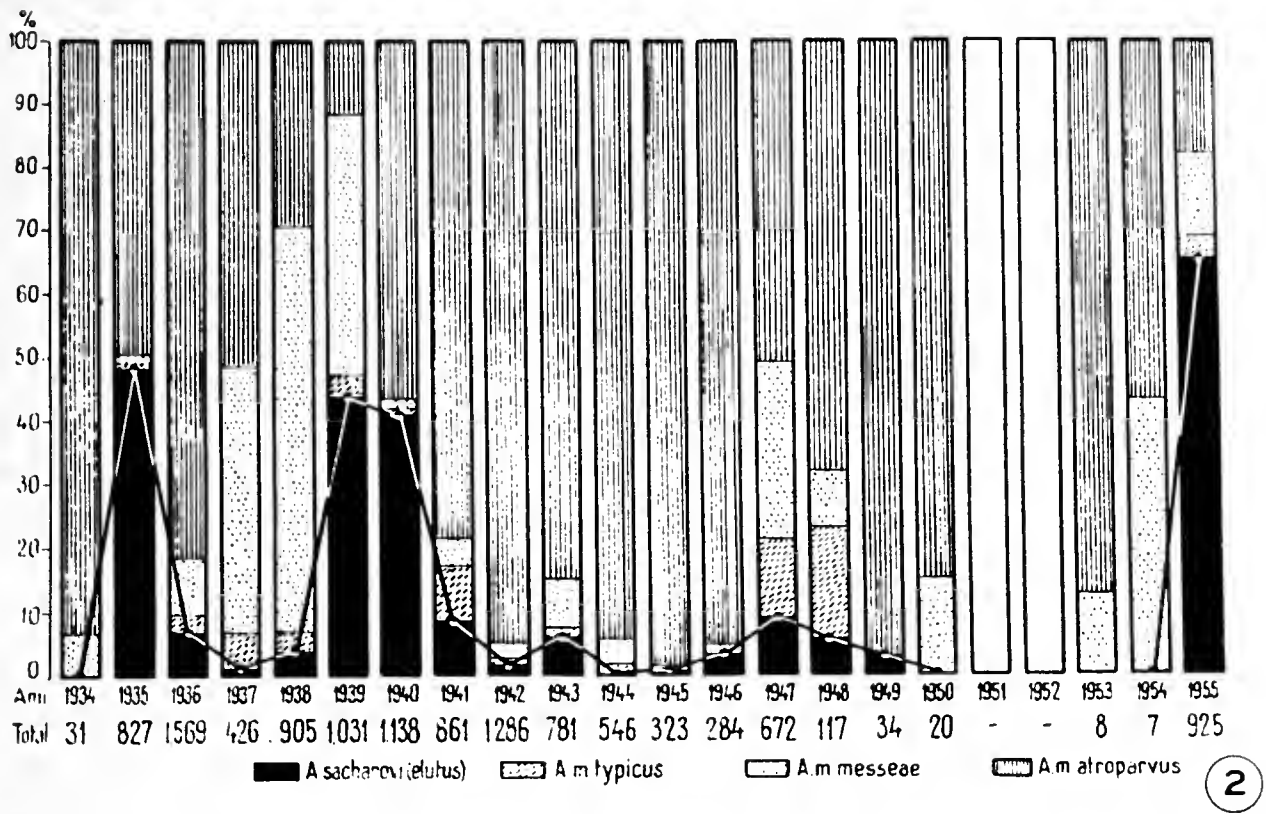
Dans nos observations pendant l'intervalle mars-octobre, les ovipositions ont été obtenues par le maintien à la température de la chambre des anophèles récoltés dans la nature et isolés par la suite; pendant l'intervalle novembre-février inclusivement, les ovipositions ont été obtenues des anophèles capturés en pleine nature et maintenus dans la chambre insectaire à la température de 27°–28° et 75–80% d'humidité. Les anophèles ont été gorgés journallement sur des lapins pendant 5–7 jours, ensuite isolés toujours dans la chambre insectaire.

En analysant les données obtenues dans une des quelques localités où les observations ont eu un caractère de continuité, on remarque de grandes variations mensuelles de l'image anophélienne au courant de la même année, par exemple: à Agigea (1935: conditions naturelles du milieu; 1936: commencement des travaux d'assainissement; 1939: dégradation complète des travaux d'assainissement commencés en 1936): Les années 1935 et 1939, bien que pré-

Fig. 2. L'évolution annuelle de la formule anophélienne dans la localité Agigea (étables et habitations). 1954–1955.

Fig. 3. Agigea. L'évolution mensuelle de la formule anophélienne (étables et habitations).

Fig. 4. L'évolution mensuelle de l'indice de l'infection spontanée: à gauche, dans les étables et dans les habitations dans la localité Mamaia-Grădinării; à droite: dans la localité Agigea (1935) en comparaison avec la courbe de Barber et Rice pour la Macédoine grèque (1932).



sentant des formules anophéliennes très différentes pour les variétés du groupe *A. maculipennis*, ont pour l'espèce *A. sacharovi* des pourcentages très rapprochés: 47.5% et 43.5%. On remarque en même temps un certain parallélisme dans l'évolution mensuelle de cette espèce, avec l'amplitude maxima de la courbe dans l'intervalle juillet-septembre (Fig. 3).

L'année 1936, à printemps précoce, est pauvre en *A. sacharovi*: 6.7%. Le fait que, pendant les mois de mars, avril, mai 1936 *A. sacharovi* pond en pourcentages de 4.0%-15.7%, valeurs qui dépassent de beaucoup celles enregistrées pendant les mêmes mois des années 1935 et 1939, prouve une grande fréquence de cette espèce dans l'anophélisme résiduel de l'année respective.

Le commencement des travaux d'assainissement au printemps de l'année 1936 et le dessèchement au cours de la même année d'une surface larvigère étendue, a déterminé pendant les mois d'été une réduction procentuelle accentuée de l'espèce *A. sacharovi*, qui ne dépasse pas en cette année 7.4% pendant l'intervalle juillet-septembre d'amplitude maxima de la courbe normale.

Les ovipositions de *A. sacharovi* à morphologie caractéristique, utilisées pour la détermination des femelles capturées, ont présenté en général au printemps et en automne des flotteurs très réduits, tandis que pendant l'été les flotteurs étaient absents. Bien que l'on ait trouvé parfois des ovipositions exemptes de flotteurs au plus tôt le 4 mars et au plus tard le 24 octobre, leur apparition en masse est notée au printemps à la fin du mois de mai et leur disparition en masse à la fin du mois de septembre. Tard, en été, au début du mois de juillet, on a enregistré d'une manière exceptionnelle une seule oviposition à très petits flotteurs. Les ovipositions obtenues dans la chambre insectaire pendant l'intervalle novembre-février, ont présenté parfois des flotteurs très petits, d'autre fois elles ont été dépourvues de flotteurs.

#### LA FRÉQUENCE DE L'ESPÈCE *A. sacharovi* (*elutus*) DANS LES HABITATIONS ET LES ÉTABLES

Dans notre pays, les variétés du groupe *A. maculipennis* sont rencontrées plus fréquemment dans les écuries et les étables et moins souvent dans les habitations (Ciucă, *et al.*, 1951), tandis que l'espèce *A. sacharovi* se trouve tant dans les écuries que dans les habitations. Pourtant, l'*A. sacharovi* montre une préférence nette pour les habitations. (Zotta, *et al.*, 1943). Cette constatation a été faite au moment de la réactivation des femelles (mois d'avril): Mamaia-Grădinării 83.33% dans les habitations et 12.67% dans les étables et jusqu'en hiver, pendant la période de hibernation. La fréquence relative de l'espèce *A. sacharovi*, étudiée à Agigea, Mangalia-Grădinării et Mamaia-Grădinării (Fig. 5), sa constance plus élevée dans les habitations et les différences de pourcentage entre les données enregistrées pour les habitations et pour les étables, indiquent l'anthropophilie de ce vecteur. (Missiroli *et al.*, 1933).

#### L'INDEX D'INFECTION SPONTANÉE

La préférence nette pour les habitations de l'*A. sacharovi* fait que cette espèce s'infecte en de grandes proportions en présence du réservoir humain de hématozoaire. Ainsi, pendant l'année 1939 à endémicité élevée dans la zone du littoral, sur 6,103 dissections d'anophèles récoltés et déterminés d'après le caractère des ovipositions, on a trouvé: 56 anophèles infectés (oocystes et sporozoïtes) parmi lesquels 49 (87.5%) *A. sacharovi* et 7 (12.5%) *A. m. atroparvus*.

Au cours de l'année 1943, dans la zone du lac Razelm à faune anophélienne similaire, sur 763 dissections d'anophèles récoltés dans les localités Zebil, Enisala, Caramanchioi, Jurilofca, Ceamurlia de Jos), on a trouvé: 38 exemplaires infectés dont 31 (96.9%) *A. sacharovi* et 1 (3.1%) *A. m. atroparvus* (Duport, 1944).

De l'examen de ces deux exemples, il résulte que dans les localités de cette zone et en présence de la faune anophélienne mentionnée, ce sont l'espèce *A. sacharovi*, et dans une bien moindre mesure l'espèce *A. m. atroparvus*, qui constituent les principaux vecteurs du paludisme. Le rapport entre le degré d'infection de ces deux vecteurs *A. sacharovi*: *A. m. atroparvus*, de 7: 1 dans le premier exemple et de 31: 1 dans le second, confirme nettement que l'espèce *A. sacharovi* est le plus redoutable vecteur du paludisme dans cette zone.

Par les dissections effectuées mensuellement pour établir les index oocystique et sporozoïtique on constate que, en exceptant les rares cas d'anophèles infectés trouvés au printemps, pendant le mois de mars même, l'infection spontanée apparaît en général au



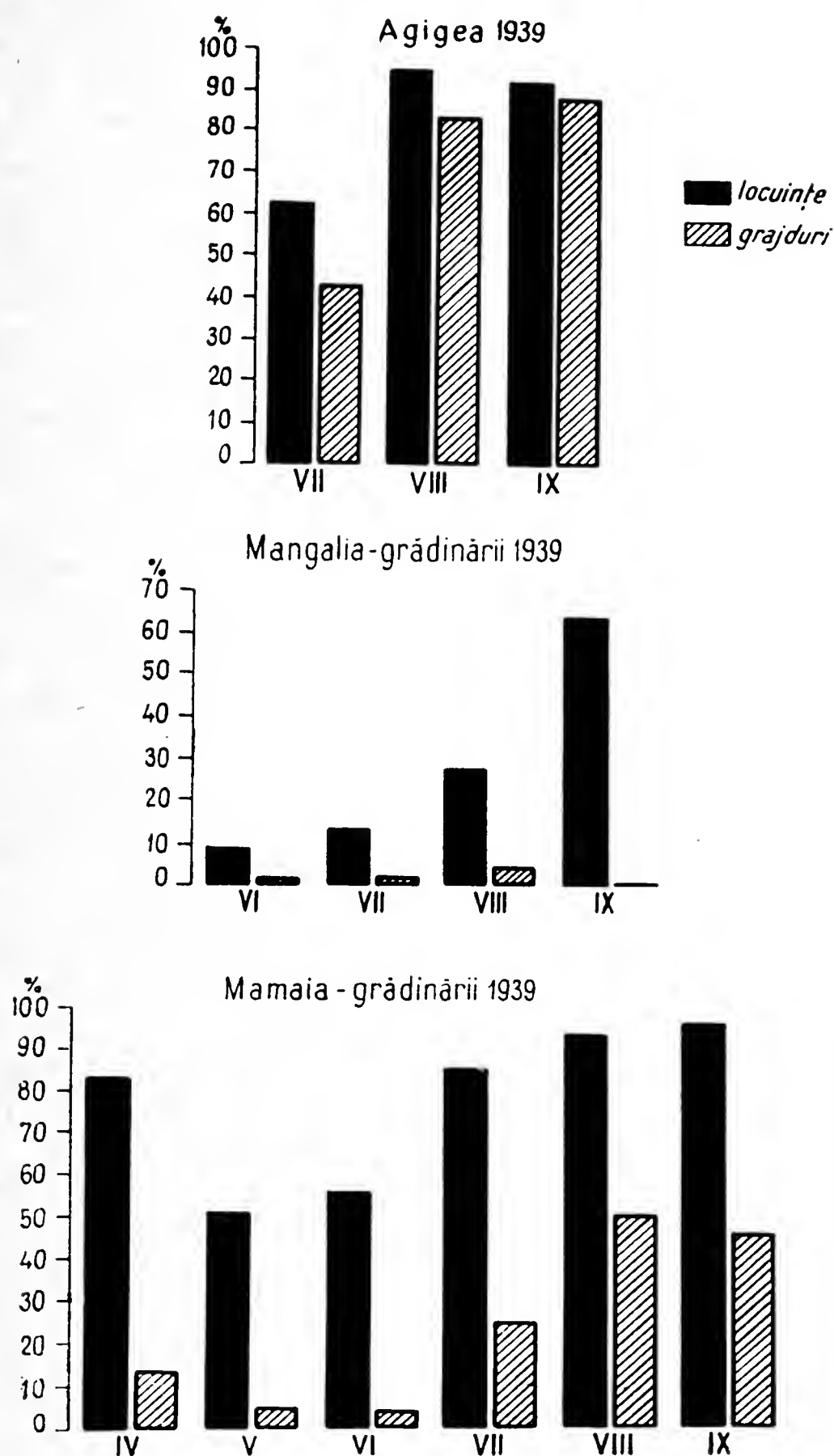


Fig. 5. La fréquence de l'espèce *A. sacharovi* dans les étables et dans les habitations.

mois de juin et de juillet. Elle croît, devenant très élevée pendant la seconde moitié de l'époque épidémique, pour continuer aussi pendant l'époque post-épidémique.

Dans nos recherches, l'index d'infection spontanée a été établi sur des anophèles déterminés d'après le caractère des ovipositions et sur ceux nondéterminés, "en vrac". Vu que l'on a constaté pour les anophèles déterminés que la positivité appartient presque exclusivement à l'espèce *A. sacharovi*, nous pouvons déduire que les anophèles infectés, disséqués du matériel "en vrac" font partie aussi, en grande majorité, de la même espèce. Ainsi, la courbe de l'évolution mensuelle de l'infection spontanée dans les localités étudiées peut être considérée comme appartenant à l'espèce *A. sacharovi*.

Entre les années 1935-1941 on a disséqué, en vu d'établir l'index oocystique et sporozoïtique 92,734 anophèles capturés dans les localités de la zone du littoral, situées en majorité entre "Două Mai" et Năvodari, en enregistrant 494 anophèles infectés. Un nombre de 356 anophèles infectés sur 44.707 exemplaires disséqués, représentant 72,1% du total des positifs, ont été trouvés dans les localités: Agigea, Techirghiol, Mangalia-Grădinării et Mamaia-Grădinării. En examinant les données concernant l'évolution de l'infection spontanée des espèces vecteurs capturées dans les habitations et les étables des 4 localités

mentionnées, il résulte que les années à grande positivité ont été 1935 pour Agigea et 1937, 1938, 1939 pour Mamaia-Grădinarii. Les valeurs enregistrées pour les collectivités Agigea (1935) et Mamaia-Grădinarii (1937, 1938, 1939) (Fig. 4) dépassent de beaucoup le niveau de la courbe de l'infection spontanée de l'espèce *A. sacharovi* étudiée par Barber et Rice en 1932, (Barber et Rice 1935, Rice et Barber 1931) sur le littoral de la mer Egée, dans la Macédoine grecque. La courbe de la localité Agigea (1935) présente un remarquable parallélisme avec la courbe du littoral de la Macédoine grecque (1932) avec un décalage de deux mois dû à la différence des conditions climatiques, générales et annuelles de notre pays.

#### L'ÉVOLUTION DE L'ENDÉMO-ÉPIDÉMIE PALUDÉENNE EN RAPPORT AVEC LES MÉTHODES UTILISÉES

A partir de 1933, date à laquelle a été signalé pour la première fois la présence de l'espèce *A. sacharovi* (*elutus*) sur le littoral, les investigations étendues d'année en année, ont montré qu'un nombre de plus en plus grand de localités était infesté de paludisme. La première vague épidémique de paludisme a été constatée en 1934 sur la portion du littoral entre Mangalia et Constanța (Tableau I).

TABLEAU I—Investigations épidémiologiques—1934. Littoral de la Mer Noire.

Localité	Population	Date	Nombre des enfants	Examen hemat. posit.	Index plasmodique	Spleno-mégalias	Index splénique
Agigea	330	12.X.1934	124	73	56.82	68	55
Techirghiol	2,034	"	272	99	40	38	11.60
Limanul	1,538	13.X.1934	372	214	57.14	211	56
Două Mai	630	"	138	43	35.50	81	58.70
Mangalia	3,955	30.XI.1934	592	102	17.23	135	22.80

En 1938 on constate l'extension vers le nord des vagues épidémiques d'un caractère extrêmement grave, vagues qui recouvrent le reste du littoral et les localités situées entre le Bras Sf. Cheorghe et le complexe des lacs Razelm-Sinoe. (Zotta 1938). Dans la majorité de ces localités, l'incidence varie entre 60–90%, quand à l'espèce *A. sacharovi* (*elutus*) il se rencontre en proportion de 31–100% dans la formule anophélienne locale.

Les mesures de lutte antipaludique instituées dans cette zone pendant la période des années 1935–1938 ont été constituées par la chimiothérapie des malades et la chimioprophylaxie des groupes sélectionnés, l'utilisation des moyens larvicides (Vert de Paris et pétrole), mesures limitées d'assainissement de la zone Mangalia, Techirghiol et Agigea ainsi que l'introduction de poissons larvivores (*Gambusia affinis* et *holbrocki*).

A la suite de ces mesures, l'endémie paludéenne entre les localités Mangalia et Constanța, au commencement d'un caractère très grave "holoendémique" tend à baisser vers la fin de l'année 1938. Dans la zone du littoral et dans la zone du complexe des lacs Razelm-Sinoe, la vague épidémique croît et les investigations effectuées par les stations de lutte antipaludique Cogevalac et Zebeil, créées en 1943, nous démontrent ce phénomène (Cantacuzino et Lupașcu, 1944, Duport 1944, Săndulescu 1944, Trifon 1944) (Tableau II).

TABLEAU II—Investigations épidémiologiques.

Localité	Population	Date	Enfants examinés	Index splénique.	Index plasmodique	Index endémique Ross
Sinoe	2,683	6.X.1943	220	74.09	51.81	82.72
Pandurul	1,507	16.IX.1934	161	44.09	45.34	65.21
Zebil	1,900	20.IX.1943	150	30.67	18.67	41.33
Ceamurlia J.	1,883	24.IX.1943	150	38.67	12	44.67
Caramanchioi	1,263	28.IX.1943	150	62	78.67	90.67
Enisala	922	29.IX.1943	109	44.95	55.97	69.72
Sarichioi	4,500	29.IX.1943	150	66.66	10.33	76.33

Dans la période de la deuxième guerre mondiale (1941–1944) (Zotta, *et al.*, 1943, Cantacuzino et Lupaşcu, 1944) et pendant les années immédiatement suivantes (1945–1947) on a enregistré une exacerbation de l'endémie palustre comme conséquence directe des effets désastreux de la guerre, exacerbation illustrée par le déclenchement de la vague hyperépidémique de paludisme à *P. falciparum* de 1945–1946 dans le département de Tulcea. (Ciucă, *et al.*, 1948). Un premier sondage systématique effectué au cours du IV-ème trimestre de 1946 a montré la gravité du problème du paludisme dans le département de Tulcea, où, à l'exception de quelques localités, l'index parasitaire de la population (de 0–14 ans) s'élevait à plus de 50%, atteignant 70% dans les localités Malcoci, Carasuhatul de Jos, Beibugeac, Murighiol et Congaz. L'index parasitaire pour le groupe d'âge de 0–14 ans variait entre 25.63% (Dunavățul de Jos) et 94.54% (Beibugeac).

En plein hiver, au cours du mois de janvier 1947, sur 1,023 examens hémathologiques, on a constaté 510 cas positifs où la proportion du *P. falciparum* était supérieure à 80% (80.89%). L'examen de 16,011 frottis de sang pour la recherche du hématozoaire palustre avec une proportion de 83.91% d'infections au *P. falciparum* et une mortalité de 210/100,000 habitants (1946), marquait le caractère fulminant de l'épidémie au *P. falciparum* dans le département de Tulcea.

La zone la plus atteinte s'étendait le long du bras Sf.Gheorghe du Danube, à partir de l'est de la ville de Tulcea et jusqu'à Dunavăț, continuant plus au sud jusqu'à Ceamurlia de Jos et à Lunca. Dans le Delta du Danube, où le paludisme constituait autrefois une rareté, un contrôle postépidémique effectué en 1946 à Sulina, a montré un index plasmodique de 29.12%, tandis que les infections au *P. falciparum* étaient représentées en proportion de 66.83% du total des examens positifs. Pour entraver la vague hyperépidémique et pour réduire la mortalité par le paludisme, le Ministère de la Santé a entrepris l'organisation d'une action systématique de chimiothérapie et de chimioprophylaxie, en utilisant sur une vaste échelle les progrès réalisés en ce domaine sur le plan mondial, ainsi que l'expérience des malariologues roumains. (Ciucă, *et al.*, 1944, Ciucă, *et al.*, 1948, Ciucă, *et al.*, 1950, Ciucă, *et al.*, 1951).

A cette première action de thérapie en masse appliquée au cours des années 1947 et 1948, s'ajoute, à partir de 1948, l'utilisation des insecticides remanents hydrocarburochlorés (DDT et HCH). (Ciucă, *et al.*, 1951). Les résultats favorables obtenus dans la première étape expérimentale montraient que l'utilisation d'une seule méthode (chimioprophylaxie et chimiothérapie) ne peut tout de même pas réduire l'endémie paludéenne que jusqu'à une certaine limite. (Ciucă, *et al.*, 1948).

L'utilisation des insecticides rémanents, associée au traitement individuel des cas et la chimioprophylaxie des groupes sélectionnés a conduit à la diminution massive du paludisme, après les deux premières années d'utilisation de la méthode combinée (1949, 1950), le nombre des cas en 1952 étant extrêmement réduit (11 cas), pour que, à partir de 1953, on n'enregistre plus un seul cas nouveau de paludisme, dans toute cette ancienne zone hyperendémique. Malgré la diminution massive des densités des espèces anophéliennes dans les collectivités pulvérisées avec des insecticides hydrocarburochlorés, nos observations ont démontré la persistance de l'espèce *Anopheles sacharovi* (*elutus*) dans cette zone. Les tests effectués pour contrôler l'instauration éventuelle du phénomène de résistance ou, éventuellement, seulement la diminution de la sensibilité aux insecticides nous ont montré que, dans les conditions de notre pays et avec les insecticides que nous avons utilisés, des modifications dans la sensibilité des espèces anophéliennes par rapport aux insecticides n'ont pas apparu jusqu'à présent. L'absence, jusqu'à présent, d'une résistance acquise par les vecteurs de cette zone en rapport avec les insecticides est confirmée par le test épidémiologique: la réduction et le maintien de la morbidité à des valeurs très réduites et même jusqu'à 0 dans toute la zone.

## CONCLUSIONS

Des conditions de milieu—des facteurs orohydrographiques et climatiques—favorables au développement de l'anophélisme et à l'entretien de l'endémioépidémie palustre, se rencontrent sur une surface de près de 30% du territoire de la R.P.R.; la variation de ces facteurs détermine le caractère de la faune anophélienne et implicitement de l'endémie palustre.

Les espèces anophéliennes identifiées jusqu'à présent dans notre pays sont: *A. maculipennis*, avec les variétés *A. m. typicus*, *A. m. messeae* et *A. m. atroparvus*, *A. sacharovi* (*elutus*), *A. hyrcanus*, var. *pseudopictus* (*A. sinensis*), *A. clavier* (*bifurcatus*) et *A. plumbeus* (*nigripes*). Parmi ces espèces anophéliennes, les variétés du groupe *A. maculipennis* et *A. sacharovi* (*elutus*) sont les vecteurs du paludisme. Les espèces et les variétés vecteurs sont rarement rencontrées en populations pures ou presque pures; le plus souvent, elles sont associées en proportions variées dans la formule anophélienne d'une localité. *A. sacharovi* (*elutus*), espèce caractéristique du climat méditerranéen et de l'Asie Mineure, trouve dans notre pays des conditions favorables à son développement seulement sur le littoral de la Dobroudja, zone qui représente la limite nord de diffusion de cette espèce.

Les observations effectuées avec continuité à partir de 1933 quand elle a été signalée pour la première fois, ont précisé que l'aire de répartition de cette espèce, à l'est de la Dobroudja, comprend une bande de 12–15 km. le long du littoral marin, à partir de la frontière méridionale de la Dobroudja jusqu'à la localité Corbul de Sus, où elle s'éloigne de la mer entre laquelle s'interpose la complexe des grands lacs Razelm-Sinoe; elle avance vers le nord jusqu'à Tulcea, port danubien approximativement à 60 km. ouest de la Mer Noire et jusqu'à Sulina, dans le Delta, sur la zone du littoral marin.

Les limites d'expansion sud et nord sont représentées par les localités Două Mai et Sulina, situées approximativement à 160 km. l'une de l'autre. Un seul point de pénétration à l'intérieur, isolé comme une île, est représenté par les localités Medjidia et Castelu, situées à 35–40 km. ouest du littoral. L'image anophélienne d'une localité ou d'une zone n'a de valeur que pour l'année et l'époque quand on a effectué les captures, étant donné que les variations peuvent se produire d'une année à l'autre et même au courant de la même année.

L'absence d'un vecteur pendant une ou plusieurs années ne l'élimine point de la potentialité anophélienne d'une localité ou d'une zone où il a été identifié antérieurement, tant que n'interviennent pas des modifications importantes dans l'orohydrographie locale; sa présence en une seule année indique son aire de répartition qui reste valable dans les conditions mentionnées. L'activité de l'espèce *A. sacharovi* au cours d'une année revêt dans notre pays les aspects suivantes: les femelles hibernantes commencent généralement leur activité hématophage, l'activité gonotrophique et le vol d'abri en abri plus tard que les variétés du groupe *A. maculipennis*. Tandis que les ovipositions pour l'*A. m. typicus*, *A. m. messeae* et *A. m. atroparvus* s'obtiennent dès le mois de mars, pour l'espèce *A. sacharovi* la déposition des pontes est plus tardive, commençant au mois d'avril. Exceptionnellement, pendant les années aux printemps précoces et chauds on obtient au mois de mars aussi des ovipositions de cette espèce. Pendant les mois d'avril, mai, juin, on obtient un pourcentage réduit d'ovipositions de l'espèce *A. sacharovi*, pour enregistrer au mois de juillet une brusque croissance. L'intervalle juillet–septembre est caractérisé par les pourcentages les plus élevés et même par la prédominance de cette espèce dans certaines localités. A la fin du mois de septembre et au début du mois d'octobre commence la baisse de l'activité gonotrophique avec la brusque réduction des dépositions de pontes, plus tôt que pour le groupe *A. maculipennis*.

Dans notre pays, l'espèce *A. sacharovi* hiberne complètement pendant les mois d'hiver, les femelles s'abritant tant dans les écuries que dans les habitations. Les déterminations effectuées pendant l'hiver (dans des conditions d'élevage) montrent que cette espèce est présente dans la maison ainsi que dans les étables. La présence en nombre comparativement plus élevé de l'espèce *A. sacharovi* dans les étables en cette période, n'indique pas nécessairement la préférence pour ce type d'abri pendant l'hiver, mais plutôt plus de possibilités de retraites et de conservation que dans les habitations.

Les variétés du groupe *A. maculipennis* sont rencontrées fréquemment dans les étables et plus rarement dans les habitations, tandis que l'espèce *A. sacharovi* se rencontre dans les écuries tout comme dans les habitations. Toutefois, dans les conditions de notre pays, l'*A. sacharovi* montre une préférence nette pour les habitations. Cette constatation a été faite du moment de la réactivation des femelles résiduelles (au mois d'avril) et jusqu'en hiver, pendant la période de hibernation.

La fréquence relative de l'espèce *A. sacharovi*, constamment plus élevée dans les habitations, et les différences procentuelles entre les dates enregistrées pour les habitations et pour les étables, indiquent l'anthropophilie de ce vecteur. La préférence nette de l'espèce *A. sacharovi* pour les chambres habitées détermine son infection dans des proportions



beaucoup plus grandes que les variétés du groupe *A. maculipennis*. Le rapport d'infection variant entre 87.5%–96.9% pour l'*A. sacharovi* et 12.5%–3.1% pour *A. atroparvus*. Les valeurs enregistrées pour l'index d'infection spontanée dépassent dans certaines localités celles enregistrées par Barber et Rice en Macédoine (1935).

La courbe de l'évolution mensuelle peut présenter quelquefois le même parallélisme, mais avec un décalage de deux mois, dû à la différence des conditions climatiques générales et annuelles de notre pays. La présence de cette espèce sur le littoral de la Dobroudja a déterminé, à partir de 1934–1935, des vagues épidémiques de plus en plus graves, culminant dans les années 1945–1946 quand on a enregistré une mortalité de 105–210 pour 100,000 habitants.

L'utilisation des insecticides rémanents, associée au traitement individuel des cas et à la chimioprophylaxie des groupes sélectionnés, a conduit à la diminution massive du paludisme après les deux premières années d'utilisation de la méthode combinée (1949–1950), le nombre des cas en 1952 étant extrêmement réduit (11 cas) pour que, en 1953, on n'enregistre plus aucun cas de paludisme dans cette zone hyperendémique. Malgré la diminution massive des densités des espèces anophéliennes dans les collectivités pulvérisées avec des insecticides hydrocarburochlorés, nos observations ont montré la présence continue de l'espèce *A. sacharovi* (*elutus*) dans cette zone.

Les tests faits pour contrôler l'éventuelle instauration des phénomènes de résistance ou, éventuellement seulement la diminution de la sensibilité aux insecticides (Farid, 1954, Garrett-Jones et Gramiccia, 1954, Livadas et Georgopoulos, 1953) nous ont montré que, dans les conditions de notre pays et des insecticides que nous avons utilisés, n'ont pas apparus jusqu'à présent des modifications dans la sensibilité des espèces anophéliennes par rapport aux insecticides. L'absence, jusqu'à présent, d'une résistance acquise contre les insecticides chez les vecteurs de cette zone est confirmée par le test épidémiologique: la réduction et le maintien de la morbidité à des valeurs très basses et même jusqu'à 0, dans toute la zone.

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## DISCUSSION

H. GALLIARD. Un fait intéressant à constater c'est la persistance d'*Anopheles sacharovi* après la disparition du paludisme. N'y a-t-il pas un vecteur plus important que cet *Anopheles* qui aurait été détruit?

G. LUPASCU. Il ne s'agit pas d'une variété différente d'*A. sacharovi* (*elutus*). Sa présence en Roumanie a été accompagnée par des fortes vagues épidémiques. L'utilisation d'insecticide remanentes comme le traitement des cas ont permis des grands succès et la baisse de la morbidité par la malaria. Nous avons montré que l'*A. sacharovi* persiste, qu'il maintient ses habitudes, qu'il ne présente pas des modifications de sensibilité à l'égard des insecticides utilisées. En absence de réservoir hématozoaire, il ne peut plus s'infecter. Le problème de l'éradication est de bien surveiller la population—d'une manière active—pour découvrir en temps utile chaque cas, tenant compte du fait que le vecteur existe et que nous ne pouvons utiliser à l'infini les insecticides, pour ne pas favoriser l'apparition du phénomène de résistance aux insecticides.

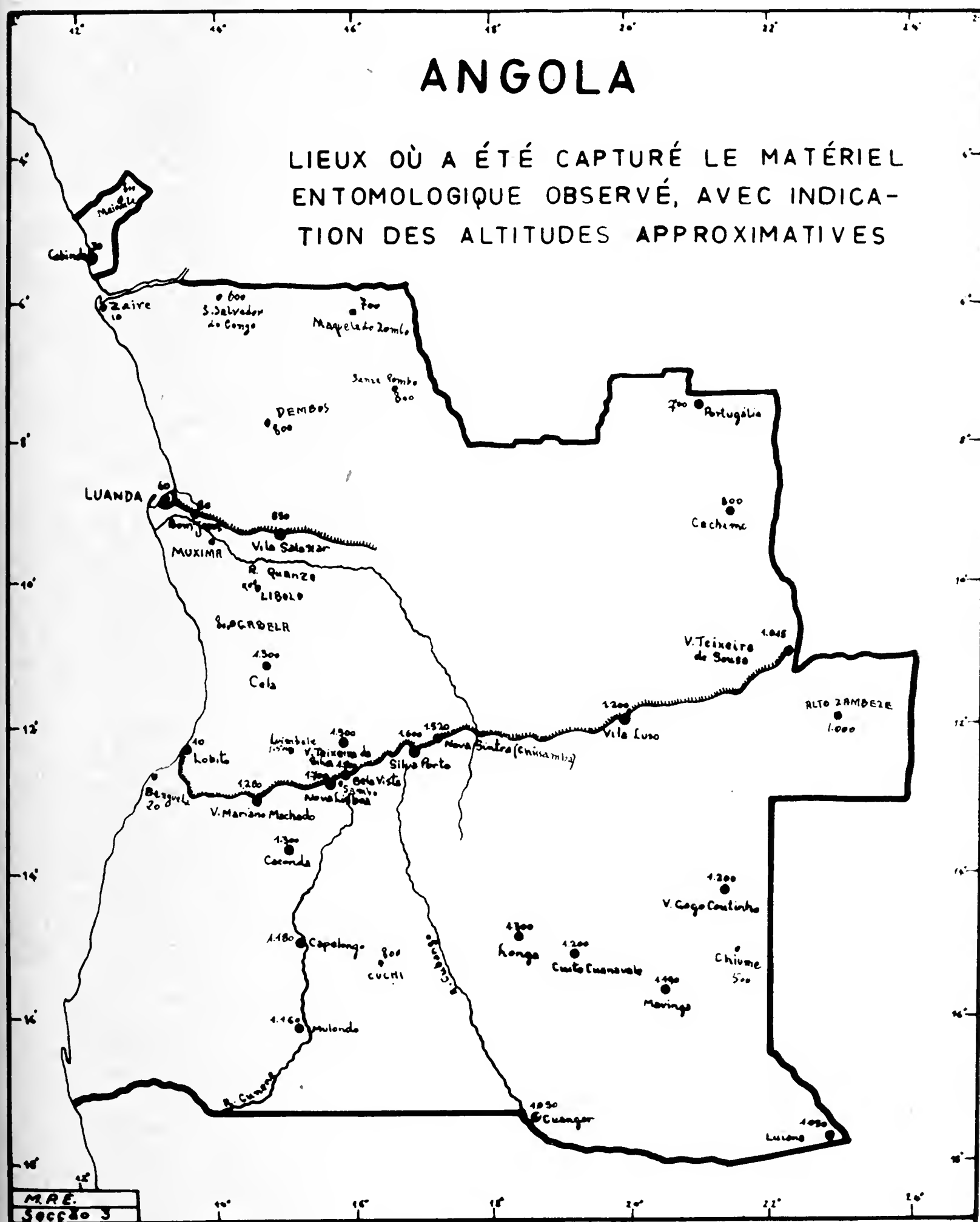
## Contribution pour la Connaissance des Culicidae d'Angola

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## ABSTRACT

During work in Angola by field sections of the "Missão de Prospeccção de Endemias" some thousands of specimens of Culicidae (early and imagal stages) were collected. These were studied to identify the species and to ascertain their distribution in the province of Angola.

Les premières références dont nous avons connaissance sur les Culicidae d'Angola remontent à 1903. Elles sont dues à Antonio Bernardino Roque qui a fait mention des espèces *Anopheles superpictus*, *Culex pipiens*, *C. elegans*, *C. malatiae*, et *C. annulatus*. Les autres



travaux sur le sujet sont dus à Frederick C. Wellman qui a recueilli beaucoup de matériel à Angola de 1903 à 1905, lequel a été étudié par lui et par Theobald. Firmino Sant'Anna, en 1920, a fait l'étude de quelques Culicidae du territoire et en 1942 et 1952 Bruno de Mesquita s'est occupé du sujet. En 1952, Colaço a fait aussi l'étude de la question dans quelques régions d'Angola.

En tant que contribution pour la connaissance du sujet, j'ai réuni durant quatre ans, de 1952 à 1955, quelques exemplaires de Culicidae du territoire dans les régions mentionnées dans la fig. ci-jointe. De cette façon, il m'a été possible d'étudier 4,750 exemplaires, soit 1,097 larves et 1,122 adultes de la tribu Anophelini et 1,827 larves et 704 adultes de la tribu Culicini, dont j'ai moi-même fait la classification, surtout pendant mon séjour à l'Institut Pasteur, à Paris, pendant les mois de juin et juillet 1955, auprès de Jacques Hamon, dont l'aide a été pour moi très utile. Pour cela, j'ai utilisé surtout les travaux de base de Theobald, d'Edwards et d'Hopkins pour la tribu Culicini et de Botha de Meillon pour la tribu Anophelini.

En plus des espèces qui seront ensuite mentionnées il y en a d'autres dont il n'a pas été possible de faire la classification jusqu'à cette date. Pour quelques-unes d'entre elles il s'agit certainement d'espèces nouvelles dont la classification exige une étude plus approfondie et une récolte plus grande de spécimens, ce que nous avons l'intention de réaliser.

Les espèces qu'il m'a été possible de classer sont les suivantes:

#### I. TRIBU ANOPHELINI

*Anopheles (A) coustani* Laveran 1900 var. *tenebrosus* Donitz 1902.

Adultes: 13 exemplaires, 1 en Mulondo (rives du Cunene) et 12 près de Cuchi (rives du Cueleï).

A. (A) *coustani* var. *ziemanni* Grunberg 1902.

Adultes: 270 exemplaires, à Bom Jesus (Catete), Capelongo (rives du Cunene), Caconda (Colonato Indígena), Cuando-Cubango, Lobito, Mulondo (rives du Cunene), Vila Gago Coutinho (Bundas) et Vila Salazar (Cazengo).

A. *Myzomyia ardensis* Theobald 1905.

Larves: 1 exemplaire à Caconda (Colonato Indígena).

A. (M) *argenteolobatus* Gough 1910.

Larves: 4 exemplaires à Bailundo et 40 à Cuando-Cubango.

Adultes: 5 exemplaires à Vila Luso.

A. (M) *barberellus* Evans 1932.

Adultes: 1 exemplaire à Vila Gago Coutinho.

A. (M) *funestus* Giles 1900.

Larves: 16 exemplaires à Caconda (Colonato Indígena), Capelongo (rive du Cunene), district de Cuando-Cubango, Lobito (près de l'aéroport) et à Vila Gago Coutinho (Bundas).

Adultes: 40 exemplaires à Bela Vista (Nova Lisboa), Bom Jesus (Catete), Lobito (zone de l'aéroport), Portugalia (Lunda), Vila Salazar (Cazengo), Cuchi (rive du Cueleï).

A. (M) *gambiae* Giles 1902.

Larves: 370 exemplaires à Bailundo, Capelongo, Lobito (zone de l'aéroport), Luanda (aéroport), Nova Lisboa et Mulondo (rive du Cunene).

Adultes: 590 exemplaires à Caconda (Colonato Indígena), Capelongo (rive du Cunene), Cela, Lobito (aéroport), Luanda (aéroport), et Mulondo (rive du Cunene).

A. (M) *gambiae* var. *melas* Theobald 1903.

Larves: 170 exemplaires près de l'aéroport de Lobito.

A. (M) *harperi* Evans 1936.

Larves: deux larves à Caconda.

A. (M) *listeri* De Meillon 1931.

Larves: 53 larves près de l'aéroport de Lobito.

Adultes: 8 exemplaires (aéroport de Lobito).

A. (M) *marshalli* Theobald 1903.

Adultes: 14 exemplaires à Portugalia et Cachimo ainsi qu'à Lunda.

A. (M) *multicolor* Cambouliu 1902.

Larves: 9 exemplaires à Lobito.

A. (M) *natalensis* 1907.

Larves: 6 exemplaires à Caconda (Colonato Indígena).

A. (M) *njombiensis* Peters 1955 (17).

Adultes: 1 exemplaire femelle, à Nova Lisboa.

A. (M) *pharoensis* Theobald 1901.

Larves: 32 exemplaires (aéroport) près de Luanda, et deux également à Lobito (aéroport).

Adultes: 23 femelles, Lobito, Luanda.

A. (M) *ruarinus* Edwards 1940.

Adultes: 1 exemplaire à Mulondo (rive du Cunene).

A. (M) *rufipes* Gough 1910.

Larves: 34 exemplaires, Capelongo (rive Cunene), Caconda (Colonato Indígena), Cuchi (rive du Cueleï), Ganda (Vila Mariano Machado).

Adultes: 1 femelle près du Cueleï.



- A. (M) *squamosus* Theobald 1901.  
Larves: 100 exemplaires à Capelongo (rive du Cunene), Cuangar (rive du Cubango), vallée du Lomba (Mavinga), Longa, Luiana, Luanda (aéroport), Cuando (Missão Católica do Huambo), Vila Luso (aéroport), et Mulondo (rive du Cunene).  
Adultes: 30 exemplaires à Capelongo (rive du Cunene), Cuangar (rive du Cubango), Cuchi (rive du Cueleï), Cuito, Cuanavale (district de Cuando-Cubango), Luanda (aéroport), Mavinga (Chitengue), Mulondo (rive du Cunene) et Vila Gago Coutinho (Bundas).
- A. (M) *tchekeledi* De Meillon e Leson 1940.  
Adultes: 3 exemplaires (femelles) sur la rive du Cuito Cuanavale, Angola.
- A. (M) *walravensi* Edwards 1930.  
Adultes: 120 exemplaires à Vila Gago Coutinho (Bundas).

EN PLUS DE CES ESPÈCES, VOICI CELLES QUI ONT ÉTÉ DÉCRITES PAR LES AUTEURS MENTIONNÉS:

- Anopheles* (*Anopheles*) *obscurus* Grunberg 1905. Mentionné par Bruno de Mesquita à Bailundo.
- A. (A) *paludis* Theobald 1900. Mentionné par Bruno de Mesquita à Alto Zambese, Bailundo, Maiombe Maquela do Zombo et Zaire.
- A. (M) *austeni* Theobald 1905. Capturé par Wellman et Fay au Bié.
- A. (M) *brunnipes* Theobald 1910. Capturé par Alwen Evans au Bié.
- A. (M) *hargreavesi* Evans 1927. Capturé par Bruno de Mesquita, Angola.
- A. (M) *maculipalpis* Giles 1902. Capturé par Wellman, Bailundo, Feijó Colaço à Sambo (Nova Lisboa).
- A. (M) *marshalli* var. *pitchfordi* Giles 1904. Capturé par Firmino Sant'Anna à Angola.
- A. (M) *nili* Theobald 1904. Capturé par Wellman et W. E. Fay à Angola.
- A. (M) *pretoriensis* Theobald 1903. Capturé par Bruno de Mesquita à Sanza Pombo.
- A. (M) *wellcomei* Theobald 1904. Capturé par Wellman et W. E. Fay au Bié.

## II. TRIBU CULICINI

- Aedes* (*Aedimorphus*) *argenteopunctatus* Theobald.  
Adulte: 1 femelle capturée en Mulondo (fleuve Cunene).
- A. (A) *dentatus* Theobald.  
Adultes: 2 femelles au même endroit.
- A. (A) *hirsutus* Theobald.  
Adultes: 1 femelle à Luanda (aéroport).
- A. (A) *irritans* Theobald.  
Adultes: 28 exemplaires à Luanda ainsi que dans la zone de l'aéroport.
- A. (A) *leesoni* Edwards.  
Adultes: 1 femelle, aéroport de Lobito.
- A. (A) *minutus* Theobald.  
Adultes: 7 femelles, aéroport de Lobito.
- A. (A) *natronius* Edwards.  
Larves: 1 exemplaire, aéroport de Lobito.
- A. (A) *ochraceus* Theobald.  
Adultes: 1 femelle (fleuve Cunene à Mulondo).
- A. (*Banksinella*) *circumluteolus* Theobald.  
Larves: 6 exemplaires (fleuve Cunene), Mulondo.
- A. (*Dunnius*) *albomarginatus* Newstead.  
Adultes: 2 femelles (fleuve Cunene), Mulondo.
- A. (*Finlaya*) *luteostriatus* Robinson.  
Larves: 4 exemplaires, fleuve Cueleï, Cuchi.
- A. (*Mucidus*) *scatophagoides* Theobald.  
Adultes: 3 femelles, fleuve Cunene à Mulondo.
- A. (*Stegomyia*) *aegypti* Linnaeus.  
Larves: 1 exemplaire, Vila Luso (Moxico).  
Adultes: 2 exemplaires, femelles, Vila Teixeira de Sousa (Dilôlo), et à Capelongo (fleuve Cunene).
- A. (S) *luteocephalus* Newstead.  
Adultes: 2 femelles, fleuve Cunene, à Mulondo.
- A. (S) *metallicus* Edwards.  
Adultes: 13 femelles, aéroport de Luanda. Également 1 femelle à Mulondo (fleuve Cunene).
- A. (S) *vittatus* Bigot.  
Adultes: 4 femelles près des habitations en différents endroits.
- Culex* (*Culex*) *annulioris* Theobald. } Pas de distinction à l'état larvaire.
- C. (C.) *aurantapex* Edwards. }
- Larves: 28 exemplaires, à Caconda (Colonato Indigena), Capelongo (fleuve Cunene), Chissamba (Nova Sintra) et à Vila Teixeira de Sousa (Dilôlo).
- C. (C) *argenteopunctatus* Ventrillon.  
Larves: 5 exemplaires à Cuando (Missão Católica do Huambo—Nova Lisboa).
- C. (C) *decens* Theobald. } Pas de distinction à l'état larvaire.
- C. (C) *invidiosus* Theobald. }
- Larves: 40 exemplaires, à Cueleï (Cuchi) et à Vila Teixeira de Sousa (Dilôlo).  
Adultes: 1 femelle provenant de Capelongo.
- C. (C) *duttoni* Theobald.  
Larves: 970 exemplaires. 98 de Bailundo, 62 de Caconda, 4 de Capelongo, 21 de Chiume, 2 de Chissamba, 3 de Mavinga, 18 de Cuando (Missão Católica do Huambo), 730 provenant de la zone de l'aéroport de Vila Luso (Moxico), 25 de Vila Salazar et 7 de Vila Teixeira de Sousa.

- C. (C) *ethiopicus* Edwards. } Pas de distinction à l'état larvaire.  
 C. (C) *bitaeniorhynchus* Giles. }  
 Larves: 10 exemplaires à Lobito (aéroport) et à Mulondo, près du fleuve Cunene.
- C. (C) *fatigans* Wiedemann.  
 Larves: 14 exemplaires provenant de Capelongo (fleuve Cunene), Cuando (Missão Católica do Huambo) et de Vila Luso (aéroport).  
 Adultes: 5 femelles (aéroport de Lobito).
- C. (C) *grahami* Theobald.  
 Larves: 1 exemplaire (aéroport de Lobito).
- C. (C) *pipiens* Linnaeus.  
 Larves: 1 femelle, Capelongo (fleuve Cunene).
- C. (C) *poecilipes* Theobald.  
 Larves: 100 exemplaires provenant de Capelongo (fleuve Cunene), Luanda (aéroport), Mulondo (fleuve Cunene) et de Mavinga (district de Cuando-Cubango).  
 Adultes: 40 exemplaires de Capelongo et Mulondo.
- C. (C) *simpsoni* Theobald.  
 Larves: 550 exemplaires.  
 Adultes: 30 exemplaires (aéroport de Luanda).
- C. (C) *telesilla* De Meillon et Lavoipierre.  
 Larves: 11 exemplaires de Capelongo (fleuve Cunene) et de Vila Luso (Moxico).
- C. (C) *thalassius* Theobald.  
 Adultes: 34 exemplaires femelles, à Julho (aéroport de Lobito).
- C. (C) *theileri* Theobald.  
 Larves: 2 exemplaires à Vila Gago Coutinho.
- C. (C) *univittatus* (Theobald).  
 Adultes: 15 exemplaires à Caconda. Egalement une femelle.
- C. (C) *watti* Edwards.  
 Adultes: 14 exemplaires à Vila Salazar (Cazengo). 1 femelle à Caconda (Colonato Indígena) et 1 autre à Portugalia, Lunda.
- C. (C) *zombaensis* Theobald.  
 Larves: 20 exemplaires à Capelongo (fleuve Cunene), Chissamba (Nova Sintra) et à Mulondo (fleuve Cunene).
- C. (*Culiciomyia*) *cinerellus* Edwards.  
 Larves: 2 exemplaires à Mulondo.
- C. (*Lutzia*) *tigripes* Grandpré.  
 Larves: 23 exemplaires de Caconda (Colonato Indígena), Capelongo (fleuve Cunene), Vila Mariano Machado (Ganda), Cuando (Missão Católica do Huambo), Vila Luso (Moxico) et Vila Salazar (Cazengo). 1 femelle également à Capelongo.
- C. (*Mochtogenes*) *inconspicuus* Theobald.  
 Larves: 5 exemplaires à Caconda (Colonato Indígena).
- C. (*Neoculex*) *horridus* Edwards.  
 Larves: 2 exemplaires (fleuve Cuelel), Cuchi.
- Taeniorhynchus* (*Mansonioides*) *africanus* Theobald.  
 Adultes: 30 exemplaires de Luanda (aéroport) et du fleuve Cuelel, Cuchi.
- T. (M) *uniformis* Theobald.  
 Adultes: 470 exemplaires à Bom Jesus (Catete), Cela, district de Cuando-Cubango, Lobito (aéroport), Luanda (aéroport), Mulondo (fleuve Cunene), Vila Gago Coutinho (Bundas), et Vila Mariano Machado (Ganda).
- Uranotaenia balfouri* Theobald.  
 Larves: 31 exemplaires, Lobito.

EN PLUS DES CULICINI, LES ESPÈCES SUIVANTES ONT ÉTÉ DÉCRITES PAR LES AUTEURS MENTIONNÉS :

- Aedes* (*Aedimorphus*) *alboventralis* Theobald. Mentionné par Edwards dans le Bié.
- A. (A.) *marshalli* Theobald. Mentionné par Edwards en Angola et par Feijó Colaço à Sambo.
- A. (*Banksinella*) *lineatopennis* Ludlow. Mentionné par Edwards dans le Bié.
- A. (*Finlaya*) *wellmani* Theobald. Mentionné par Theobald dans le Bié et par Feijó Colaço à Nova Lisboa (Huambo).
- A. (*Stegomyia*) *africanus* Theobald. Mentionné par Theobald, fleuve Kukema à Chissamba, Nova Sintra (Bié).
- A. (S) *pseudonigeria* Theobald. Mentionné par Theobald à Huambo et Bailundo.
- A. (S) *schwetzi* Edwards. Mentionné par Feijó Colaço à Sambo.
- A. (S) *simpsoni* Theobald. Mentionné par Theobald au Bié et par Feijó Colaço à Nova Lisboa.
- Culex* (*Culex*) *guiarti* Blanchard. Mentionné par Wellman à Angola.
- C. (*Culiciomyia*) *nebulosus* Theobald. Mentionné par Feijó Colaço à Sambo.
- Eretmapodites chrysogaster* Graham. Mentionné par Edwards à Cabinda.
- Ficalbia* (*Etorleptiomyia*) *mediolineata* Theobald. Mentionné par Feijó Colaço à Luanda.
- F. (*Ficalbia*) *uniformis* Theobald. Mentionné par Feijó Colaço à Sambo.
- Taeniorhynchus* (*Coquilletidia*) *cristatus* Theobald. Mentionné par Edwards à S. Salvador do Congo.
- T. (C.) *fuscopennatus* Theobald. Mentionné par Theobald à S. Salvador do Congo.
- T. (C.) *metallicus* Theobald. Mentionné par Theobald et Edwards et désigné par "*Chrysoconops nigra*", en Angola.
- T. (C.) *nigritorax* Theobald. Mentionné par Theobald et désigné par "*Chrysoconops nigrithorax*" à Luimbale.
- Género *Theobaldia*. Mentionné par Bernardino Roque à Angola et désigné par "*Culex annulata*".

De cette façon on vérifie qu'il remonte à 30 le nombre d'espèces d'*Anopheles* décrites jusqu'à cette date en Angola et à 57 le nombre de Culicini.

### APPRÉCIATIONS FINALES

Cette étude globale sur les Culicidae d'Angola qui vient d'être présentée ne peut pas cependant être considérée comme parfaite. En réalité, puisqu'il s'agit d'un territoire aussi vaste que l'est l'Angola, le sujet exige une étude plus approfondie, ce que nous avons d'ailleurs l'intention de réaliser. J'ai considéré cependant comme du plus grand intérêt de présenter à ce Congrès ce qu'il m'a été possible de réunir sur un sujet si important, en considérant le rôle de quelques espèces de Culicidae dans la transmission d'assez de maladies en Afrique et également à Angola.





# Biting Midges (Ceratopogonidae) as Intermediate Hosts for *Haemoproteus* of Ducks

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## ABSTRACT<sup>1</sup>

Many domestic ducks that were placed out-of-doors during June and July in each of the years 1954 to 1956 in Algonquin Park, Ontario, became infected with *Haemoproteus nettionis* (Johnson and Cleland), although species of Hippoboscidae were never taken from, nor observed on, any of them. Certain species of mosquitoes, black flies (Simuliidae), and biting midges (Ceratopogonidae) were known to feed on the ducks at the time they became infected with *Haemoproteus*. A series of observations led to the conviction that Culicoides is an intermediate host of *H. nettionis*. This was proven by producing infections in nine of eleven ducks that were injected with suspensions of biting midges. Some of the suspensions consisted of midges that were collected at night in a light-trap, others were of specimens that were kept ten and eleven days after their engorgement at night on infected ducks. Natural transmission of *Haemoproteus* also occurred during the night, indicating further that certain midges fed at this time. The species of Culicoides that was taken most often from ducks was never noticed feeding on man. Structures thought to be developing oöcysts and sporozoites were seen in sections of specimens of Culicoides that had fed previously on infected ducks.

## DISCUSSION

I. B. TARSHIS. 1. Were developing stages of *Haemoproteus* found in the flies? 2. Were sporozoites found in the salivary glands, or oökinetes in the gut wall of the flies? 3. Is this a new species of *Haemoproteus*?

R. C. ANDERSON. 1. Dr. Fallis has observed the early stage in the flies, but it is hoped to study later stages in sections of preserved material. 2. As far as I know Dr. Fallis has not observed sporozoites in salivary glands, but oökinetes have been observed. I do not know where the oökinetes observed by him were located. 3. In his paper Dr. Fallis refers to the *Haemoproteus* in domestic ducks in Algonquin Park as "*Haemoproteus nettionis*".

<sup>1</sup>Paper will be submitted to the *Canadian Journal of Zoology*.



# Sur l'Occurrence de la *Glossina palpalis palpalis* dans l'Île du Prince (Province Portugaise de S. Thomas et Prince - S. Tomé e Príncipe)

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## 1. DESCRIPTION GÉOGRAPHIQUE

L'île du Prince constitue une partie de la province portugaise de S. Tomé e Príncipe. Elle se situe dans le golfe de Guinée entre les parallèles de 1°32' et 1°41' Lat. N et les méridiens 7°19' et 7°27' E. Mer. G. (Fig. 1). Sa superficie totale est de 126 km<sup>2</sup>, sa longueur maximum étant de 17 km, alors que sa largeur maximum est de 10 km. La côte de l'île est très irrégulière, tantôt plate, tantôt accidentée.

Le sol est d'origine volcanique et se présente très accidenté, particulièrement dans le tiers méridional, où s'observent d'importants et de nombreux massifs montagneux. La végétation de l'île (Fig. 3) est très exubérante surtout dans la partie sud qui est en grande partie formée encore par la forêt vierge. Une chaîne de montagnes bien détachée sépare les 2/3 du nord de l'île du tiers sud. A peu près les 2/3 de l'île, ceux du nord, sont bien cultivés surtout pour le cacao, la production principale de l'île, les autres cultures étant représentées par le café, le coco et l'huile "demdem" (*Elaeis guineensis*), sans compter la culture d'arbres fruitiers, dont les principaux sont le bananier et l'arbre du fruit-pain. L'île présente de nombreux cours d'eau et plusieurs marécages qui sont encerclés en général par une végétation exubérante.

Le climat de l'île est du type équatorial avec une moyenne thermique annuelle de 25°, 4 et de faibles oscillations de température qui ne vont pas au cours des différents mois de l'année au-delà de 3°; les oscillations journalières maximales ne vont pas au-delà de 7°, 5. L'humidité relative est assez élevée, généralement entre 70 et 80%. En ce qui concerne les saisons, il y a une saison sèche bien marquée, connue localement par la saison de la *gravana*, allant de juin à septembre, et une autre saison pluvieuse, allant d'octobre à mai, avec une interruption correspondant à la petite saison sèche ou au *gravanito*, de janvier et février. La pluviosité annuelle est pour la partie sud de 3.000 mms par année, tandis que pour la partie nord elle est de 2.000.

L'île est peuplée par des Africains qui sont venus du continent, ce qui forme la population autochtone, et par des travailleurs venant périodiquement d'autres territoires et particulièrement dans les dernières années des îles du Cape Vert. Pour une population totale de 4.000 habitants il y a à peu près 2.500, soit 62.5% de travailleurs et 1.500, soit 37.5% autochtones; le nombre d'Européens est à peu près de 150.

## 2. RÉSUMÉ HISTORIQUE DE LA MALADIE DU SOMMEIL DANS L'ÎLE

L'île du Prince a souffert beaucoup de la maladie du sommeil vers la fin du dernier siècle et le commencement de l'actuel par suite de l'invasion du territoire par la mouche tsé-tsé. L'île a été découverte en 1471 par les Portugais João de Santarem et Pedro Escobar, le territoire se trouvant alors inhabité. Elle se peupla surtout vers la moitié du dernier siècle par les Africains venant de la partie occidentale de l'Afrique et plus particulièrement du Gabon, étant donné que l'île a été un important entrepôt pour les Africains qui alors se dirigeaient vers l'Amérique. On suppose que c'est au cours de la seconde moitié du dernier siècle que la maladie a été introduite dans l'île et avec elle la mouche tsé-tsé.

En 1871 la première description de la maladie fut faite dans l'île et on suppose que la *Glossina* y a été introduite vers 1825 puisqu'à cette époque il y avait des relations commerciales très fréquentes entre l'île et le continent africain, établies par des bateaux. Les conditions que les glossines ont trouvées dans l'île pour leur développement ont été des meilleures et de cette façon on comprend bien pourquoi la maladie a acquis dans l'île un caractère épidémique de la plus haute importance, puisque la mortalité par la maladie a atteint 22.1% en 1901, tandis que les infectés en 1907 étaient de 26.0%. Il semblait vraiment qu'on ne pouvait pas habiter l'île puisque, alors qu'en 1885 la population autochtone était

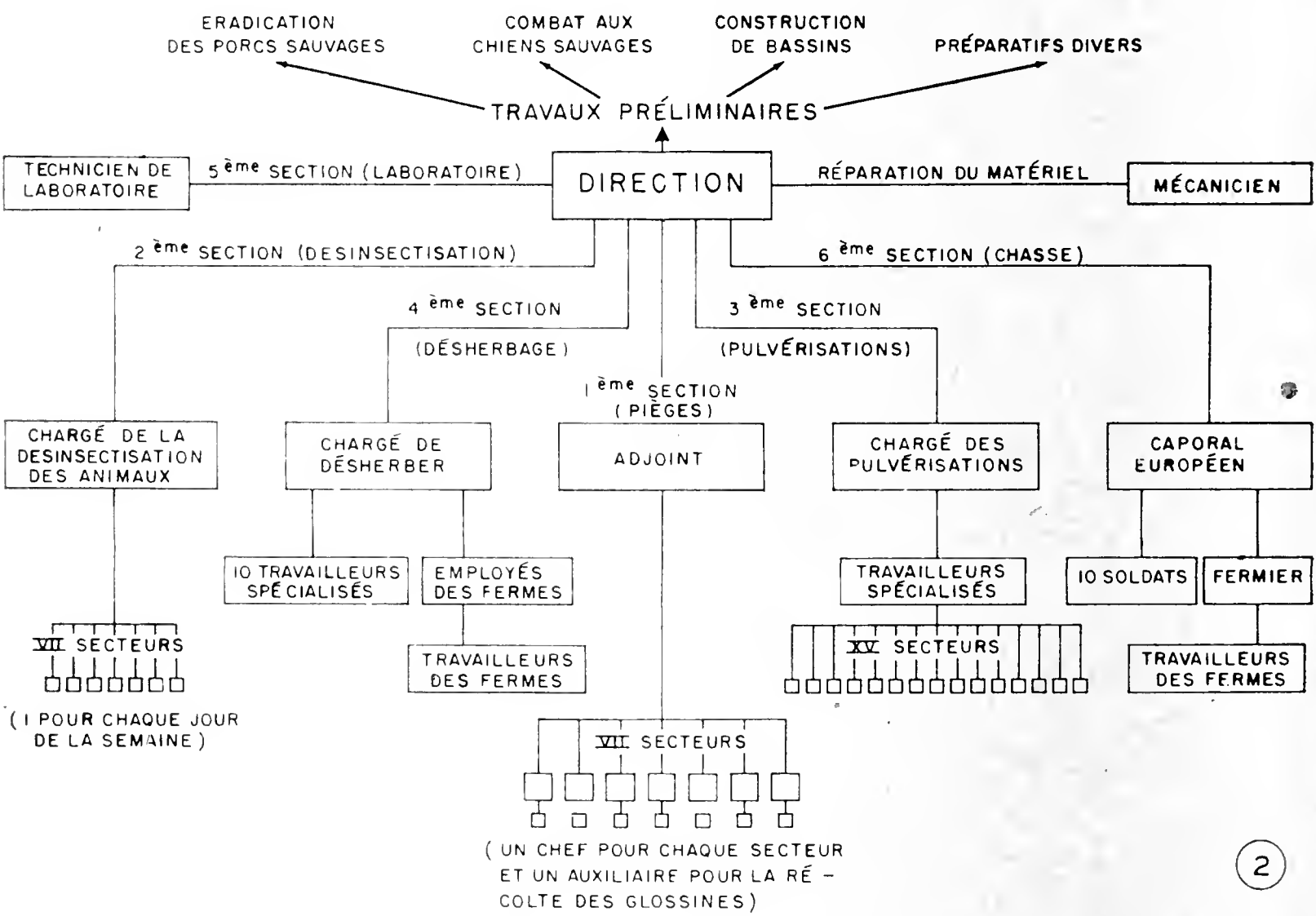
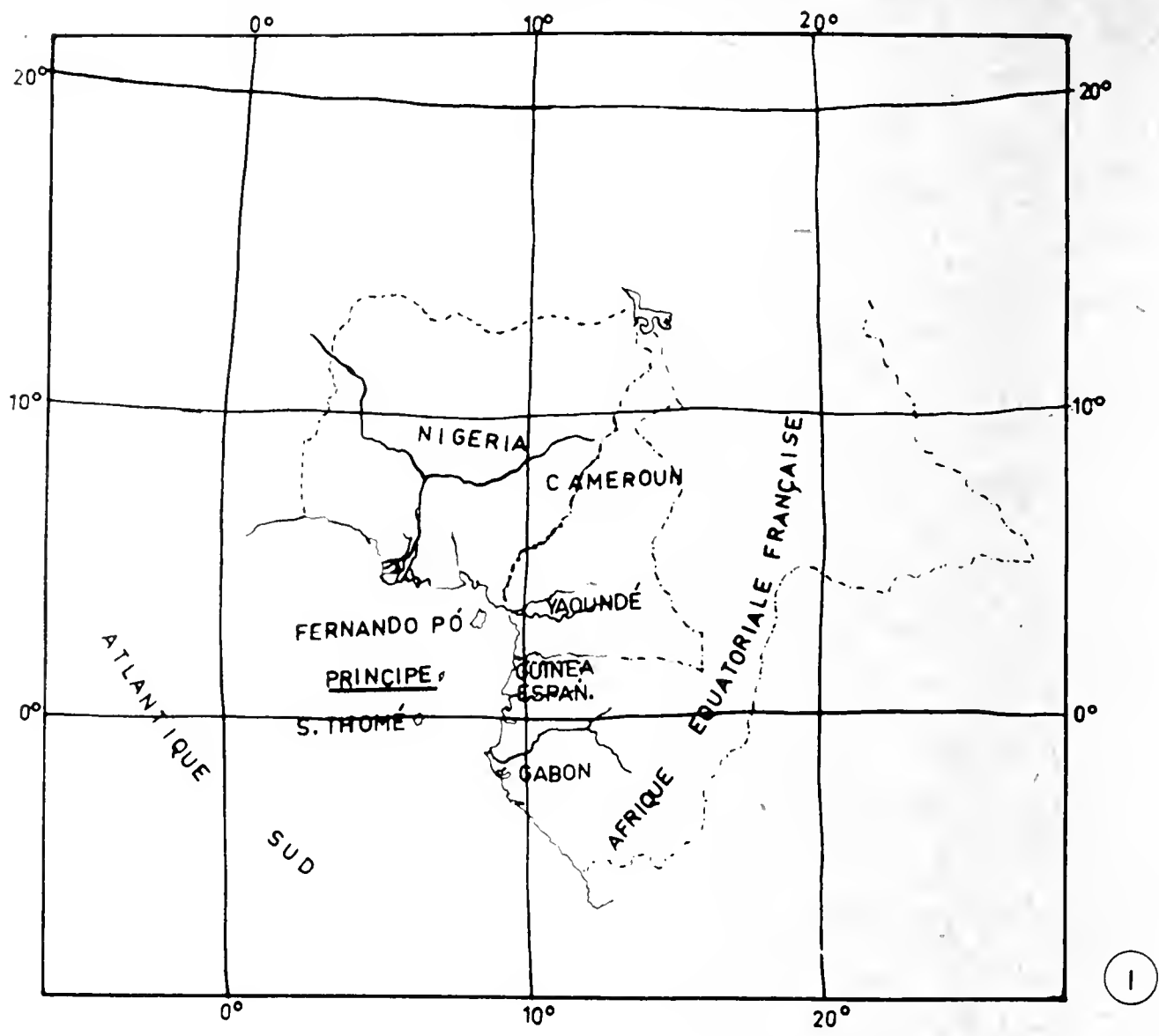


Fig. 1. Situation géographique de l'Ile du Prince.  
Fig. 2. Schème du plan de lutte contre les glossines.

de 3,000 habitants, en 1900 elle était seulement de 800 et en 1907 de 350. Par suite d'une mortalité si élevée, on suggéra au gouvernement portugais l'abandon définitif du territoire. Cependant, le traitement de la maladie du sommeil par l'atoxil a été découvert en 1905, et en même temps on prenait connaissance, avec des détails chaque fois plus nom-



breux, des conditions de la transmission de la maladie par les glossines. De cette façon, une lutte très intense et efficace se réalisa contre la maladie et contre les glossines, de 1911 à 1914, lutte dont le résultat aurait été l'éradication de ces insectes de l'île. En avril 1914, la dernière glossine fut capturée dans le territoire.

Nous pensons pouvoir admettre que les glossines ont été exterminées pour les raisons suivantes:

1. Le système de contrôle des glossines à l'occasion de la campagne de 1911-1914 a été maintenu jusqu'en 1915 (7 juin) sans qu'aucune mouche soit capturée depuis avril 1914.
2. La population locale qui a si bien connu les glossines ne les avaient jamais remarquées après cette date.
3. Au mois de décembre 1932 Tams, du Musée Britannique, a fait pendant un mois la capture d'insectes dans l'île sans trouver de glossines.
4. En 1954 et 1955, des zoologues de la Junta de Investigações do Ultramar ont travaillé dans l'île sans y trouver de glossines.

Cependant, le 3 mai de cette année (1956), quelques exemplaires de mouches ont été reçus dans la Junta de Investigações do Ultramar, envoyées de l'île et considérées suspectes. L'examen des exemplaires fait par un de nous (Tendeiro) a révélé qu'il s'agissait de la *Glossina palpalis palpalis*. Devant cette constatation si importante, l'étude du problème s'imposait, et dans ce sens une mission d'étude spéciale a été organisée par l'Instituto de Medicina Tropical et par la Junta mentionnée ci-dessus; la mission partit pour l'île le 13 du même mois de mai. Ce sont les résultats de ses premiers travaux que nous venons présenter à ce Congrès.

### 3. LA DISTRIBUTION DE LA GLOSSINE DANS L'ILE

Le premier objectif de la mission était de vérifier la présence de maladies du sommeil et d'animaux infectés par les trypanosomes. Dans ce sens, on fit l'examen clinique et par le laboratoire de toute la population de l'île et d'un grand nombre d'animaux domestiques avec des résultats négatifs. La mission a fait aussi jusqu'à cette date, 1er juillet, la dissection de 3.000 glossines avec des résultats négatifs.

Cependant, depuis le début de ses travaux, la mission a étudié la distribution des glossines dans l'île, en essayant d'abord pour cela la même méthode d'application de matière visqueuse qui avait été employée par la mission de 1911-1914 et qui est connue comme la méthode "Maldonado", et en utilisant également des pièges des modèles Harris et Morris. Ce sont les pièges Morris qui se sont révélés les plus efficaces. En essayant les pièges Morris, nous avons expérimenté différents types de tissus de revêtement et aussi l'introduction dans quelques-uns d'entre eux d'un porc comme animal-piège. Ce sont les pièges avec revêtement de tissu noir qui se sont révélés les plus efficaces. Les pièges avec porc capturaient davantage de spécimens, mais les difficultés de leur emploi ne justifiaient pas leur utilisation.

Pour connaître la distribution et l'incidence des glossines dans l'île, nous avons installé en différents endroits des groupes de 10-12 pièges Morris et y avons compté les exemplaires capturés au cours d'une semaine. Le nombre total de glossines capturés par les dix ou 12 pièges divisé par 10 nous donnait ce que nous avons désigné par "piège-semaine". De cette façon, par les nombres correspondant aux pièges-semaines des différents endroits, nous avons pu évaluer la densité de distribution des glossines et préciser quels étaient les endroits les plus favorables pour leur développement.

### 4. LE PLAN DE LUTTE CONTRE LA GLOSSINE EN VUE DE SON ÉRADICATION

Bien qu'il n'y ait pas de maladies du sommeil, d'animaux avec trypanosomes, ou de glossines infectées, l'éradication de ces insectes s'impose étant donné son danger potentiel, et dans ce sens le plan suivant a été adopté et est mis à exécution (Fig. 2).

#### (A) EXTERMINATION DES COCHONS SAUVAGES

Dans l'île il y a beaucoup de cochons qui vivent en liberté soit parce qu'ils se sont enfuis, soit parce qu'il y a des entreprises agricoles qui les élèvent en pleine liberté. Etant

donné que les glossines aiment beaucoup à faire leurs repas sur les cochons, il est naturellement indiqué de les exterminer pour supprimer une telle source d'alimentation. A part les cochons, il y a très peu d'animaux sauvages sur lesquels les glossines peuvent se nourrir, puisqu'il y a seulement de très rares civettes (*Viverra civetta* Schreber), animaux aux habitudes nocturnes, et par conséquent très peu recherchés par les glossines, et des singes (*Cercopithecus mona mona* Schreber), considérés également comme étant très peu favorables aux glossines. Il y a encore quelques chiens domestiques devenus sauvages qui seront aussi tués. Les autres petits animaux, comme les petits lézards et les oiseaux, ne comptent pas.

#### (B) UTILISATION DES ANIMAUX DOMESTIQUES COMME MOYEN DE DESTRUCTION DES GLOSSINES

Dans ce but, ces animaux seront soumis chaque semaine à des bains d'insecticides d'action résiduelle. A cet effet, l'île sera divisée en des secteurs et dans chacun seront construits des installations pour des bains anti-parasitaires, de deux genres: un grand pour les animaux de grandes taille, tels que les bœufs, les chevaux, les mules, et les ânes; l'autre plus petit pour les autres animaux tels que les cochons, les chèvres, les chiens, et les moutons. Comme tous les animaux sont enregistrés, il est facile de vérifier si tous sont ou non baignés. Les animaux de chaque secteur se dirigeront vers le territoire correspondant de façon à ce que, dans la mesure du possible, les glossines du secteur puissent s'y nourrir, ce qui aura pour résultat leur mort par suite du contact avec l'insecticide recouvrant l'animal.

#### (C) UTILISATION DES PIÈGES MORRIS

On fera une large distribution de ces pièges de façon à totaliser au moins 6,000 dans toute l'île qui sera divisée pour cela en secteurs. Les pièges seront aussi imprégnés par un bain d'insecticide de façon à ce que toutes les glossines qui viendront se reposer doivent également mourir. Jusqu'au premier juillet 925 pièges ont été installés, lesquels ont capturé 50,131 mouches (Tableau I).

TABLEAU I. Glossines Capturées depuis le Commencement du Travail.

Semaines	Période	Glossines capturées	Glossines capturées dans les pièges	Total des pièges installés	Pièges semaine
1ère	15-20/V	480	409	14	29.2
2ème	21-27/V	2988	2899	182	15.9
3ème	28- 3/VI	7639	7605	304	25.0
4ème	4-10/VI	10838	10838	432	25.0
5ème	11-17/VI	9223	9223	462	14.3
6ème	18-24/VI	10038	10038	794	12.6
7ème	25- 1/VII	8925	8925	925	9.6
		50131			

#### (D) DESTRUCTION DE TOUTE LA VÉGÉTATION SECONDAIRE

Cette végétation qui s'est développée dans les endroits (Fig. 4) les moins soignés pour la culture sera détruite puisqu'elle forme généralement une protection très importante pour les glossines. Puis de tels endroits seront traités par insecticides sous la forme de pulvérisation chaque 15 jours, vu que cette période est ce qui correspond à peu près à la période entre chaque ponte.





Fig. 3. Un aspect du centre de l'Ile du Prince.

Fig. 4. Végétation secondaire ("capoeira") dans l'Ile du Prince.

#### (E) DESTRUCTION DE LA VÉGÉTATION PLUS DENSE

Elle se fera de chaque côté des fleuves et des marécages lorsque ce sera jugé nécessaire.

### 5. CONSIDÉRATIONS FINALES

Quarante-deux ans après que l'île du Prince ait été libérée des glossines, elle fut de nouveau envahie par ces insectes, probablement importés de l'île de Fernando Pó, à 200 km. de distance, reliée par transports aériens deux fois par mois. On sait que ces avions sont traités par pulvérisateurs avec le mélange habituel d'insecticide et de Pyrethre, mais cette application n'a peut-être pas été faite toujours avec la rigueur voulue.

Dans le plan de combat qui a été établi contre les glossines en vue de leur éradication, on a envisagé l'intérêt qu'il y aurait à ne pas troubler quelques situations biologiques de l'île comme nous le mentionnons ci-après:

(a) Les cocos ont été fortement envahis par une espèce de cochenille, la *Criptognatha nodiceps*, ce qui a réduit énormément la production de copra. Sous la direction de la Junta de Investigações do Ultramar, un prédateur a été importé pour l'île, en provenance de l'île de la Trinidad, l'*Aspidiotus destructor*, qui s'est adapté très bien dans l'île et qui causa la destruction presque totale de la cochenille. Pour ne pas troubler ce genre de lutte biologique, on a pensé à utiliser les insecticides avec la plus grande prudence, étant donné que le prédateur est sensible au DDT et certainement aux autres insecticides d'action résiduelle (Tableau II).

TABLEAU II. Essai sur la Résistance de l'*Aspidiotus destructor* au DDT.

Après le contact pendant 10 <sup>s</sup> avec l'Insecticide	Témoins (Sans contact)
1.30 H— 1% avec paralysie	Aucune sans manifestations
3.30 H— 80% avec paralysie	" " "
4.00 H— 20% mortes	" " "
5.00 H— 50% mortes	" " "
8.30 H— 80% mortes	" " "
10.00 H—100% mortes	" " "

(b) La fertilisation du cacao est faite par l'intermédiaire d'insectes qui font le transport du pollen, ce qui nous faisait aussi utiliser les insecticides avec les plus grandes restrictions et en des lieux limités. D'ailleurs, nous ne croyons pas que l'utilisation des insecticides sur une grande échelle, par exemple par avion, pourra conduire à des résultats efficaces, étant donné la grande densité de végétation de l'île, ce qui ne permettra pas leur accès au lieu de repos et d'activité des glossines.

Et voici ce que nous considérons le plus important à communiquer à ce Congrès sur l'introduction récente de la *Glossina palpalis palpalis* dans l'île du Prince. Nous attendons maintenant le résultat de l'exécution des plans de lutte qui ont été étudiés, plans qui ont besoin d'être éclectiques étant donné les difficultés que nous offre encore actuellement l'éradication des glossines. En même temps, cette nouvelle invasion de l'île du Prince par les glossines doit encore attirer notre attention sur la possibilité de la diffusion des glossines et d'autres insectes par les avions, et sur le besoin de ne pas oublier toutes les mesures de défense à prendre avec ces moyens de transport si rapides, mesures qui sont d'ailleurs bien connues et accessibles.



# Progress and Problems for the Future of Medical and Veterinary Entomology

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## ABSTRACT

*The control of malaria and other diseases by residual insecticides, mosquito control by area application of insecticides, human lice control by insecticides, and the protection of man from mosquitoes, mites, and other arthropods by repellents are outstanding developments during the past two decades. Poisoned baits and insecticide-treated cords are current developments that aid in the control of insecticide-resistant house flies. Water soluble or water miscible insecticides in irrigation water are promising economical means of controlling mosquito larvae. Some of the materials effective against medically important arthropods are also highly effective against important animal parasites. Progress in cattle grub control by systemic insecticides may solve this most costly insect pest problem. The use of sexually sterile male screw-worm flies in eradicating this pest from the Island of Curacao is an outstanding and unique method of insect control. Many unsolved problems remain. Ways to prevent or overcome resistance to insecticides, new and more economical ways to control important pests and disease vectors, more information on the biology, ecology, methods of colonizing many of the species, taxonomy, and a firmer basis for appraising the hazards of chemicals used in control are some of the problems remaining which emphasize the need for continued and expanded research in medical and veterinary entomology.*

## INTRODUCTION

Progress in medical and veterinary entomology during the last fifteen years has been outstanding. The more serious vector-transmitted diseases of mankind have become of minor health significance where vector-control programs have been instituted. Important advances in veterinary entomology have also been made. Since many of the same arthropods that attack man also attack livestock, control methods developed for protecting man are often useful with slight modification for livestock.

In spite of the advances, important problems remain to be resolved and new problems are constantly arising which constitute a challenge to those who are devoting their efforts to medical and veterinary entomology. Significant contributions in this field have been made in many parts of the world by entomologists and associated specialists in the fields of chemistry, medicine, and engineering. This report on progress will be limited to a general review of some of the outstanding developments in the United States on certain arthropods made during the last 10 to 12 years.

## ADVANCES IN MEDICAL ENTOMOLOGY

The scientific break-through in dealing with vectors of human diseases came with the development of DDT and other new insecticides and repellents early in World War II. Only those familiar with the previous control procedures for vectors of malaria, typhus, and other important arthropod-borne diseases that are now readily controlled with the chlorinated hydrocarbon insecticides, and certain repellents, can fully appreciate the extent of progress that has been made.

We must recognize, however, that the mere discovery of effective insecticides and repellents does not assure success in dealing with disease vectors and pests. Prior contributions of entomologists and others on vector-disease relationships, in insect taxonomy, and on the biology and ecology of many species are of vital importance in the practical use of the chemicals available today. These aspects of the problem must not be ignored in the conduct of future research.

### MOSQUITOES

Without doubt the most important single development in mosquito control is the use of residual insecticides. This method of employing DDT for mosquito control was first investigated by Gahan *et al.* (1945). Many investigators have since shown the value of residual sprays of DDT, dieldrin, chlordane, lindane, and other materials for the control of

malaria mosquitoes. With the aid of DDT malaria has virtually been eradicated from the United States by Federal and State public-health agencies. This method now forms the basis for a much greater effort to eradicate malaria from the entire North and South American continents. The success of this effort, sponsored by the Pan American Sanitary Bureau in cooperation with the nations in the Western Hemisphere, will be watched with great interest in the years ahead. The appearance of DDT-resistant strains among the *Anopheles* mosquitoes has not been a serious factor thus far in most parts of the world. However, widespread resistance is a real possibility, and we must not relax our search for substitute materials or other approaches to malaria control.

The new insecticides have an important place in mosquito control when employed in other ways. Ground and aerial dispersion methods for applying the new insecticides to control adults in vast areas, first demonstrated by Lindquist and McDuffie (1945) and Lindquist *et al.* (1945), make it possible if the occasion demands to break the chain of disease transmission immediately. These methods are currently employed primarily as supplementary measures in programs for the control of pest mosquitoes. Research prompted by the occurrence of resistance among culicines has shown that several organic phosphorus insecticides are also effective against adult mosquitoes (Gjullin and Peters 1955, 1956).

Destruction of larvae with chemicals has been the most reliable method of mosquito control in the past. The highly efficient chlorinated hydrocarbon larvicides have made it possible to control both anopheline and culicine mosquitoes at exceedingly low application rates. In recent years some of the organic phosphorus insecticides have been found even more effective. Among those that have shown good larvicidal action are parathion, EPN, malathion, and Bayer L 13/59 (Gjullin *et al.* 1953, Yates & Lindquist 1952). Unfortunately, the high mammalian toxicity of some of the first organic phosphorus insecticides delayed their acceptance for mosquito control. However, the discovery of some phosphorus compounds with moderate to low mammalian toxicity and more experience in the safe use of the more toxic materials are stimulating greater interest in their use.

Progress has also been made in methods of applying the new larvicides. The work with granular insecticides by Whitehead (1951) in heavily vegetated areas and later by Keller *et al.* (1953) against salt marsh mosquitoes has been of great importance.

Perhaps the most promising new way to employ insecticides for the control of mosquito larvae is through the use of water-soluble or water-miscible formulations. The idea of adding larvicides to irrigation water or flowing streams has been investigated for many years. When DDT and other organic insecticides became available, there was renewed interest in this method of application. However, it was soon found that the larvicides applied as emulsions, oil solutions, or suspensions in irrigation water were adsorbed on soil or plant materials and thus failed to control larvae in the more distant parts of the flooded fields. Recent investigations by Gahan and coworkers have emphasized the value of water-soluble larvicides. Following laboratory studies (Gahan *et al.* 1955b), their results with such formulations of parathion and Bayer L 13/59 (Gahan & Mulhern 1955, Gahan & Noe 1955) are extremely encouraging. This approach to effective and economical larvicidal treatment has been aided by the automatic applicator devised by Gahan *et al.* (1955a). It has been estimated that the cost of treatment with the most effective materials under investigation will be not more than 15 cents per acre-foot of water. If further research supports the early investigations water-soluble larvicides could be of outstanding significance in mosquito control. They also have advantages when employed as conventional treatments since the use of special solvents may not be necessary.

Although chemicals will continue to play an important part in mosquito control, long-range programs should emphasize water management to prevent mosquito breeding. The research on water management carried out by the U.S. Public Health Service and the Tennessee Valley Authority (1947) has not only served as a guide in minimizing mosquito production in the vast Tennessee River water-impoundment systems but will continue to be invaluable in developing similar practices in other areas.

#### BODY LICE

The value of DDT for the control of the body louse, vector of endemic typhus, is well known. When DDT-resistant strains were reported from Korea, a 1-percent lindane dust was adopted by the U.S. Army to protect its troops and war prisoners from louse

infestations. The substitution of lindane for DDT was made possible largely because of the cooperative work of the Orlando, Florida, laboratory of the U.S. Department of Agriculture and the entomologists and medical specialists in the Department of the Army. Various dust formulations containing pyrethrum or allethrin in combination with synergists such as sulfoxide or piperonyl butoxide have been found to control this louse. Phosphorus insecticides are especially effective against body lice. The most urgent need in further work on louse control is to determine what materials can be used safely on man.

### HOUSE FLIES

None of the medically important insects have received more study during recent years than the house fly. The great hopes for a permanent solution to the house fly problem through DDT residual treatments, first demonstrated by Wiesmann (1943), were dashed by the marked resistance that this fly developed within a few years after these treatments came into use. Hopes for satisfactory substitutes for DDT among other chlorinated hydrocarbon insecticides were soon dimmed because of the resistance problem.

By 1951 research workers at Orlando had changed emphasis from residual insecticides to baits as a means of fly control. Traps containing baits were re-evaluated. Various combinations of attractants and toxicants were tried. This approach led to important advances in the control of insecticide-resistant house flies. A combination of water, syrup, and the phosphorus insecticide TEPP sprinkled on the floors or other suitable surfaces in the manner devised by Thompson *et al.*, (1953) and Gahan *et al.* (1953) represented the first major success, but the great hazard of TEPP to those preparing the liquid baits made it necessary to find a safer bait. Further study soon led to the development of dry sweetened baits which could be sprinkled in buildings where flies congregate. Phosphorus insecticides low in mammalian toxicity, particularly Bayer L 13/59, malathion, and Diazinon, were found highly effective (Gahan *et al.* 1954). Dry granular baits which can be formulated in many ways have given outstanding control in many areas and are now employed extensively. However, repeated treatments are necessary. To overcome this limitation long-lasting bait formulations and methods of using them were investigated by Bruce (1953), and such sugar baits applied in strategic places inside of buildings are now providing long-lasting control in certain areas.

Control measures inside buildings based on the resting habits of the house fly have received much attention by the U.S. Public Health Service. Cords impregnated with parathion and suspended in appropriate places were found to give good control for many months, but the hazards of parathion have delayed their use. Recently Diazinon-impregnated cords have been registered for use in the United States. It is expected that they will find their place among the various methods of fly control.

In spite of recent advances in fly control, it is not known at this time how long they will be of value. There are indications that the phosphorus insecticides now in use will also become ineffective.

Research on the use of space sprays and aerosols and new-type residual treatments is continuing. The synergists such as piperonyl butoxide, sulfoxide, and propyl isomer developed by industrial companies in the United States to increase the insecticidal activity of pyrethrum have proved of outstanding value in alleviating the fly-control problem. The synthesis of allethrin by Schechter *et al.* (1949) and the demonstration of its effectiveness by Gersdorff (1949 a & b) led to the commercial development of this pyrethrum-like insecticide. Several phosphorus insecticides possessing residual properties are being used with good results in certain areas (Hansens 1956).

### ADVANCES IN VETERINARY ENTOMOLOGY

The highly significant advances in medical entomology have contributed to progress in the field of veterinary entomology. Because of the limited financial support for research on livestock insects, progress has not been as marked as in the medical entomology field. Those engaged in research on arthropods attacking livestock are to be commended, however, for the advances that have been made.

The new chlorinated hydrocarbon insecticides were soon shown to be of outstanding effectiveness against the horn fly (Laake 1949, and Laake *et al.* 1950), a pest estimated to cost livestock growers in North America over 100 million dollars each year. DDT, toxaphene,



methoxychlor, and TDE are all highly effective in sprays or dips, and there is yet no evidence of the horn fly's resistance to these materials in spite of their extensive use for a decade.

Progress in protecting livestock from other bloodsucking flies, including the stable fly, horse and deer flies, and mosquitoes, has not been outstanding. Synergized pyrethrum gives excellent protection against all the bloodsucking species, but the protection lasts only for several hours to several days, depending on the concentration applied and weather conditions. The high cost of the treatment and the short protection period make these sprays impractical for use on range animals.

Many investigators have shown that lice on all kinds of livestock and poultry and the sheep ked on sheep can be effectively controlled with any of the new chlorinated hydrocarbon insecticides that are regarded safe to apply to livestock.

Tick control has advanced greatly during the last decade. Sprays or dips containing 0.5 percent of toxaphene, as advocated by the U.S. Department of Agriculture (Division of Insects Affecting Man and Animal 1950), or toxaphene plus a small amount of gamma BHC provide excellent control for ticks in the United States. DDT alone is not a desirable tick control agent, chiefly because it has low toxicity to engorged ticks. However, it does possess sufficient residual action against unengorged ticks to provide good protection against reinfestation. Lindane, on the other hand, is an excellent material for engorged ticks but it provides protection against reinfestation for only a short period of time. A combination of DDT and lindane has therefore proved of outstanding value as a tick-control agent.

The cattle grubs *Hypoderma lineatum* and *H. bovis* are now regarded as the most costly livestock pests in North America. There has been little progress in practical control methods during the last 25 years. Rotenone sprays, dusts, and washes are still the only materials in use. The need for repeated treatments, which must be made during the winter months, discourages their widespread use.

A wide variety of new insecticides have been tested as contact sprays by the Entomology Research Branch during recent years. None of the chlorinated hydrocarbon insecticides are effective. Certain phosphorus insecticides show more promise, in that they give control comparable with or even better than the rotenone spray. However, a new contact insecticide, regardless of its effectiveness, will not obviate the necessity for repeated spraying during the grub season.

Because of the disadvantages of conventional grub-control methods, more and more consideration is being given to the use of systemic insecticides. The laboratories of the Entomology Research Branch at Kerrville, Texas, and Corvallis, Oregon, have concentrated research on this method of control for the last 10 years. A number of materials have been found that destroy the grubs systemically. The most recent material, Dow ET-57 (the purified form of 0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate), shows promise of providing a practical solution to the grub problem. The Dow Chemical Company has also intensified research on this material in efforts to develop it for practical use.

The most interesting approach to insect control, which will be discussed by R. C. Bushland, involves the release of a preponderance of sexually sterile males of the screw-worm among the natural population. This procedure, which eradicated the screw-worm in such rapid and dramatic way on the island of Curacao in the Netherlands Antilles, might prove useful for the control of certain other insects. Factors to consider in appraising the possibilities of this approach have been discussed by Knipling (1955).

### PROBLEMS FOR THE FUTURE

The advances that have been made in medical and veterinary entomology do not warrant any feeling of complacency among those engaged in this area of research. We are confronted with many unsolved problems. Methods of control now employed for many insects are unsatisfactory or too costly. This is true for the house fly, sand flies, tabanids, cattle grubs, ticks, the stable fly, and even mosquitoes in many situations.

Resistance to insecticides is without doubt the greatest obstacle to overcome. If we could assure satisfactory results for an indefinite period of time with chemicals that have been found effective or that may be discovered in the future, there would be little justification for expanded research to find better or more economical means of control. However, the tremendous setback that we have already suffered, at least in some areas, in connection with house flies, a wide range of mosquito species, human lice, ticks, bed bugs, fleas, and



cockroaches should emphasize the need for continued and expanded research. In planning such research we must take a long-range look and concentrate our efforts on many phases of this problem if we are to maintain the progress we have made.

In taking this long look ahead, I urge first of all that we not abandon the idea of employing insecticidal chemicals. We know from experience during the last dozen years that chemicals offer effective and desirable means of controlling many of the most important vectors of diseases as well as pests. However, we should make every effort in fundamental and applied research to determine more about the interaction of insecticides and the hosts. The primary objective should be to develop chemicals or combinations of chemicals that will overcome or prevent resistance for an indefinite time.

In our research on insecticides and repellents we must find materials that provide a reasonable safety margin between the therapeutic dose and that which proves hazardous to the host. Toxicological investigations are a vital part of the development of practical pesticides, and adequate support for work in this field is essential. However, in order that mankind not be denied the use of valuable weapons in its war against insects, I urge that pharmacologists, animal toxicologists, and regulatory officials be realistic in the matter. We all want safety in the use of pesticides, but safety margins should not be unnecessarily wide. In the field of veterinary entomology there is urgent need to determine what minute amounts of residues in meat, milk, and eggs mean in terms of public hazard, and reasonable tolerances based on such findings should be established.

Research on arthropods affecting man and animals should devote more emphasis to methods of control other than the use of chemicals. Environments that are not conducive to the development of high populations should be created wherever possible. We need more research on the biology, ecology, host-parasite relationships, feeding habits, nutritional requirements, and other aspects of our important arthropods. The information obtained should be used to find ways of controlling the species through sanitation, water and land management in connection with the control of mosquito and other aquatic insects, and other practices that will destroy or create unfavorable breeding places.

The utilization of biological agents to control medically important arthropods needs further study. A full exploration of pathogens that may exist among certain species and the possible utilization of such pathogens for insect control warrant special investigation. This approach for agricultural pests is being given increased attention by workers in Canada and in the United States, but little effort has been made to explore this approach for insects of medical and veterinary importance. The possibility of altering aquatic environments to favor or maintain fish and other predators to destroy mosquitoes has not been adequately investigated.

Investigations on the behavior of insects need to be intensified. What are the factors that attract an insect to the host or the food that it consumes? What is the mechanism of attraction of one sex to the other in reproduction? I feel that those relatively unexplored fields offer possibilities for controlling some of our important insects.

The control of resistant house flies has been advanced by taking advantage of their feeding habits. The existence of a "fly-factor" attractant in foods on which the house fly has been feeding has been demonstrated. The use of insecticide-treated cords takes advantage of the fly's resting and roosting behavior. A powerful attractant for male cockroaches has been found in virgin female roaches. Important contributions have been made in determining what attracts mosquitoes to man. Progress is being made in finding out what attracts the screw-worm to wounds of animals. These are examples of the kinds of research on attractants that need to be conducted in a long-range solution to insect problems.

My final comment on problems that need to be solved if we are to make progress in certain areas of research concerns the need for methods of colonizing all the important arthropods. Research along this line has been one of the most neglected areas in the entire entomology field and its importance is not fully realized by many. By and large, workers in medical and veterinary entomology have done well in developing ways and means of rearing insects under controlled conditions. Many of the medically important insects can now be reared in unlimited numbers in the laboratory. This is one reason why progress in their field has been so rapid. Methods for the successful colonization of *Anopheles quadrimaculatus* have been of unestimable value in improving control measures for these mosquitoes and in studying malaria parasites. Methods for the mass production of body lice provided the key

to rapid progress in louse control during World War II. The possibility of controlling the screw-worm by releasing sexually sterile males would not have been explored if an artificial medium and mass rearing methods had not been available. The relatively little progress in controlling the cattle grub is no doubt due largely to the inability to obtain adequate quantities of all stages of the insect for laboratory investigations. These successes and failures illustrate the importance of work on colonization methods in meeting current problems in medical and veterinary entomology.

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## DISCUSSION

D. R. JOHNSON. At present DDT and dieldrin are the principal insecticides used for anopheline control in anti-malaria programs in many countries. With development of resistance to chlorinated hydrocarbons, what other materials can be substituted for long-lasting control? What is the longest time that residual action can be expected to persist

indoors against anopheline adults when using insecticides other than the chlorinated hydrocarbon materials?

E. F. KNIPLING. Perhaps others in the audience can provide more specific information. I feel that if resistance to chlorinated hydrocarbons should become widespread we would most likely look to the phosphates. Generally the residual action is not as long as the hydrocarbons and it might be necessary to apply them more often, or determine how to time treatments to achieve malaria control with minimum residual protection. However, some of the phosphates such as diazinon and malathion will provide considerable residual protection. Would workers at Orlando comment on their findings? Also could Dr. Pepper comment on the work of Hansens?

B. B. PEPPER. The work done by Dr. E. J. Hansens in New Jersey with diazinon shows generally that residual activity depends upon the nature of the surface to which it is applied. In some cases it actively lasted for an entire year, in others it lasted for only 3 to 4 months.

CARROLL N. SMITH. At Orlando we have no records on persistence of residual action of organic phosphorus insecticides against mosquitoes indoors. Against house flies we can expect 3 weeks residual effectiveness in barns, though sometimes control lasts longer than this. In some other parts of the country control lasts much longer. Probably no general statement of the time of residual effectiveness would be applicable over a wide geographical area. Against bedbugs and roaches control has been obtained for longer periods, but, against roaches, panels from the treated houses lost effectiveness in laboratory tests some time before the premises became reinfested.

J. D. GREGSOM. I believe, Dr. Knipling, that you mentioned a lack of residual protection in the use of BHC. In the course of tick (*D. andersoni*) control in British Columbia, we have observed that crude BHC has a much greater residual action than pure lindane.

E. F. KNIPLING. That is very interesting. We have not observed this in the States. Are you concerned with one-host or three-host ticks?

Response: three-host ticks.

A. A. KINGSCOTE. Would Dr. Knipling please enlarge, if possible, on his reference to work which has been done in connection with factors attracting mosquitoes to their hosts?

E. F. KNIPLING. I had reference primarily to work done by Canadian workers. Is Dr. Brown in the audience? I was hopeful that he might elaborate on this work. Perhaps Dr. De Long, of Ohio, could discuss the work he has done along this line.

W. ROGOFF. As Dr. Knipling has pointed out, further work on understanding the behavior of our pests relative to host attraction or sex attraction can very likely lead to entirely new approaches to the control of these pests.





# The Role of the Entomologist in Medical and Veterinary Entomology

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## ABSTRACT

*In 1955 the United States celebrated its 100th year of professional entomology. However, entomologists have had a rather significant part in the medical and veterinary fields for only about 50 years. Work of the early medical and veterinary entomologist was confined largely to insect taxonomy and biology, with some responsibility for control. Public health or health of animals programs were invariably administered by medical or veterinary doctors. Today the entomologist has a much more significant position in medical and veterinary fields. This has been brought about by the greater acceptance of all kinds of specialists by the medical and veterinary professions resulting from rapid advances in taxonomy, biology, and control of insects, and in all phases of medical and veterinary medicine. The various disciplines have become so complex that they cannot all be well understood by any one person. The future of the entomologist in these fields depends to a considerable extent on how thoroughly medical and veterinary workers entrust to him the entomological problems in their fields. The entomologist must train himself so that he can demand and obtain the confidence of these workers.*

When this topic was first suggested to me I accepted with some misgivings, as I could not even decide what the definition of a medical or of a veterinary entomologist should be. I have asked many people and have found no uniformity of definition. Let us presume that the identity of the two types of entomology is made clear by the modifiers, "medical", for the study of insects that affect man, and "veterinary", for the study of insects that affect domestic animals. Many species of insects in these two entomologies are well known to be common to both; hence the two fields overlap.

When reference is made to the various texts and general papers on these two fields of entomology, one finds that in addition to the insects, ticks and mites are also included. Likewise, many workers consider scorpions, centipedes, millipedes, and spiders as parts of these fields. It is also perfectly natural that many other arthropods occurring in and around the habitations of man and his domestic animals should also be included. Finally, the crustaceans that act as intermediate hosts for certain helminth parasites are considered part of this group. In actual practice the two entomologies include that portion of parasitology that may be broadly defined as a study of arthropods of medical or of veterinary importance. They include arthropods that either affect their hosts directly by their bites or stings, or that act as transmitting hosts for other parasites, or that annoy man and domestic animals by their presence.

Patton and Cragg (1913) point out a good reason for considering these two entomologies as separate definable entities. They make the statement: "An expert knowledge of the individual forms is required, rather than a general knowledge of the class." The word "class" refers to insects in the strict sense and needs to be cast in the plural if one accepts the addition of the other arthropods in the general field of entomology. It follows then, that a medical entomologist is one who is familiar with the species of arthropods that affect man, and a veterinary entomologist is one who is familiar with the species of arthropods that affect domestic animals. Not only must the entomologist be familiar with the arthropods, but he must also be familiar with the disease organisms transmitted by the arthropods.

From an historical point of view entomology is a relatively old subject. For instance, last year the United States celebrated the 100th year of professional entomology. Of course, significant publications on both the applied and the fundamental phases of entomology were published in the United States more than 150 years ago. In the Old World there were a number of significant scientific papers devoted exclusively to both applied and fundamental entomology published more than 200 years ago (Hagen, 1862, 1863; Horn and Schenkling, 1928-1929). However, for only a little over 50 years have entomologists had a rather significant role in the medical and veterinary fields.

Historically speaking, the early medical and veterinary entomological landmarks were established by workers trained in the field of medicine. Almost all of the classical examples of disease transmitted by arthropods such as malaria, Texas cattle fever, sleeping sickness, and the association of filariasis with mosquitoes, were discovered by men trained in medicine and not in entomology. During those early years when the medical and veterinary men were studying diseases and disease transmissions, entomologists were occupying themselves with descriptions of new species and were making biological observations on biology. Of course the medical and veterinary workers were aided materially by the facts established by entomologists. However, almost no entomologists prior to about 50 years ago were greatly interested in the role of insects in disease transmissions.

There was probably little need on the part of the medical and veterinary workers prior to 1900 to seek the aid of entomologists, for after all, entomology in those days was a rather uncomplicated subject. This same thought was pointed out by L. O. Howard (1930) when he commented on the problems of the early days: "At first medical men seemed to feel that entomology was after all a simple thing and that it would be easy for them to handle the whole field thus developed. But it has become obvious that to secure the best results, men trained in economic entomology and broadly trained in the biology of insects are of the utmost importance." Without a doubt Howard would today be willing to alter his statement to include veterinary entomology, and also to include other phases of entomology than the biology of insects and economic entomology.

Following the important discoveries of Manson, Ross, Grassi, Smith, Kilbourne and many others, as the 20th century approached and shortly thereafter, there has been a tremendous increase of interest among entomologists in problems involving disease transmission by pestiferous arthropods.

Today, the entomologist has a much more significant position in medical and veterinary fields than did the earlier workers. This shift in responsibility has been brought about in part by the increased willingness of the medical and veterinary professions to accept the help of experts in other fields of science. This willingness is largely the result of the rapid advances in taxonomy, biology, and insect control as well as in all phases of medical and veterinary medicine. At the same time, entomologists have become more interested in medical and veterinary entomology and are training themselves and directing their attentions to the study of disease transmitting arthropods.

Entomologists in the two fields must be able to teach and do research in taxonomy, biology, distribution, control, and mode of disease transmissions of insects of medical and veterinary importance. Now let me give a few examples of what the entomologists are doing along these lines.

Unfortunately, few entomologists are directing full time to the teaching of medical or veterinary entomology. In all but a few instances, the teaching positions include additional obligations in other fields. Thus their work in medical and veterinary entomology is actually on a part time basis. I am glad to say, however, that as the importance of insects affecting man and animals becomes more apparent, classroom hours devoted to these subjects are being increased.

Everyone recognizes that morphology is the basic tool used by all who identify living things; a great deal of progress is being made in this field that is of direct benefit to the medical and veterinary entomologist. One of these benefits is to standardize names of structures. For instance, Belkin (1950, 1952) has presented us with a satisfactory system for identifying the larval and pupal hairs on mosquitoes. Wharton *et al.* (1951) have done the same for mites.

As aids to taxonomy, entomologists have been testing serological methods as a means of separating species complexes. A number of papers on this subject are in the literature concerning animals other than insects. Unique in our field is a paper by Leone (1947) in which he demonstrates identifiable differences in antibodies produced in rabbits when injected with antigens of cockroaches.

Chromatographic techniques have been found to give additional aid in a more accurate identification of insects of medical importance. The works of Micks and Ellis (1952), Ball and Clark (1953) demonstrate the usefulness of this technique.

The recent work of Jucci (1947) in Italy on the chromosomal differences in the salivary glands of *Anopheles* is an example of the use of a new tool that may prove quite useful

in insect taxonomy. Especially outstanding at this conference are a number of very significant papers on the use of chromosomes as an aid in the taxonomy of complex species groups. I really have been thrilled by the papers presented here in this field.

I have been chiding my taxonomy friends by telling them that in the future we will make a paper chromatograph of an insect, throw away the fragments of the insect, and place the chromatograph on a pin in the collection. I now see we must include a slide of the chromosomes on the same pin.

Almost inseparable from the taxonomic problems are the studies on the biologies of insects. Often species complexes that are eventually unraveled by the entomologists were first recognized by differences in the biologies of the component species. In other instances, only after identifications were established were the biological differences noted.

An outstanding example of the need for more biological observation appeared when DDT first was used. This insecticide was used to kill insects that came naturally to treated surfaces. It was neither necessary to use an attractant nor to make applications directly on the insect. But entomologists found immediately that their knowledge of the resting habits of mosquitoes and flies was thoroughly inadequate. They needed to know more and more biology of the old fashioned kind.

A study of biology may not be as spectacular as some of the new chemical, physiological, and genetical studies, but it is fundamental to an understanding of a biological system. I recently asked a fellow worker what progress he was making in the colonization of black flies. He told me he had tried and had failed, but that he had spent the summer in the field trying to find why he had failed. Certainly this was a worthwhile use of his time. Until we find a method to colonize black flies we are unable to study their role in the transmission of disease organisms.

Up to the time DDT became available for public use, the control of insects of medical and veterinary importance was confined largely to one or two arsenicals, two alkaloids (pyrethrum and rotenone), and oils. While these older materials are still effective, lower dosages of newer insecticides have produced much better control. Entomologists are actually hard pressed to make adequate tests of the great number of new insecticides being developed today. In addition to the extremely low dosages and the variety of formulations, new application equipment needs to be, and is being, developed.

To add to these complications, certain insect pests have been rapidly developing insecticide resistance. As a consequence the entomologist has had to direct much of his research time towards the resistance problems. Not only is it necessary to make tests to verify resistance, but the newer materials must be tested to find those that will be effective on resistant insects.

One of the more neglected fields of study is that of geographical distribution of arthropods of medical and veterinary importance. It is encouraging to find an increase in the number of papers on this subject within recent years. I am especially sensitive to the deficiencies in this portion of entomological publication, for at Cornell we are trying to list the arthropods of medical importance for the world, itemized for each country. This work is being done for the Army Office of the Quartermaster General; after several years the lists are still quite incomplete. When completed, they should be of material aid to those visiting foreign countries, for then one can have a better idea in advance, which species of arthropods of medical importance might be encountered in any given land. We solicit your aid in this task as we want our lists as complete and as accurate as possible.

The entomologist has as his primary role in the transmission studies, the handling of insects. Those problems concerned with disease transmission can be attacked only with the cooperation of the medical and veterinary professions. There is no doubt that the events of World War II brought about a coordination of entomologists, physicians, and veterinarians at a much more rapid rate than could be expected during times of peace. This coordination came about, at least in part, because each of the concerned workers found that his obligations far exceeded his time and experience, and it became necessary to call on the proper specialists concerned.

I have now come to the part of my discussion that should be entitled, "How To Lose Friends and Alienate People". I see in this group some of my friends who may take exception to what I have to say. I can only hope that they will not cast me out as a friend and coworker. I firmly believe that the future of the entomologist in the medical and veteri-



nary entomology is quite promising. However, the maximum development in these two fields depends primarily on the amount of coordination and cooperation that the entomologists receive from the physicians and veterinarians.

The subject matter of the three disciplines represented is so complicated that no single worker can claim competence in all phases of medical or veterinary entomology. As a consequence one usually finds it necessary to consult specialists to obtain the best answers. The literature, the new techniques and the new observations in each field have progressed to a point where even the specialist cannot keep abreast of his own field, much less that of other fields. Naturally this is well recognized by all—but what can be done to assure maximum progress in all three disciplines? Several suggestions for the future development and improvement of the coordination between entomologists, physicians, and veterinarians might be made.

Perhaps the quickest way to improve coordination would be for the physicians and the veterinarians to increase their acceptance of the entomologist as a specialist who can offer material assistance in the problems involving arthropods. There often is a marked reluctance on the part of those trained in medicine and veterinary sciences to accept the entomologist as a fellow scientist. Often the medical and veterinary workers fail to entrust entomological problems to the entomologist. As a consequence, the entomologist, if he enters into the investigation at all involving arthropods, may find himself working merely as a technician, and he may find that his talents are not effectively utilized. This view is expressed by many entomologists and is well founded, as I can attest from personal experience and observation. Often the physician or the veterinarian tries to do the entomological work himself with little or no advice from the entomologist. I feel that unless the physicians or veterinarians are trained in entomology they should not waste their time with the arthropod phases of entomological problems. Instead, it seems logical that they should apply themselves to the phases of the problems for which they are trained, and rely on entomologists to handle the arthropod problems.

In spite of the currently known roles of arthropods in disease transmissions, medical and veterinary schools rarely have an entomologist on the staff. This is perhaps understandable as the curricula in these schools is already overfilled with subjects more closely allied to the requirements of the professions. There simply is no time to devote to a thorough study of arthropods. The only answer to this problem seems to be a more thorough course in medical and veterinary entomology as part of the premedical and preveterinary training.

The entomologist, of course, is to be criticized for not training himself along medical and veterinary lines if he wishes to work in these fields. Often the entomologist has such a poor academic background in medical and veterinary fields that he does not deserve the confidence of the medical and veterinary workers. With minor changes in teaching, a program of a strong training in entomology for premedical and preveterinary students would meet the approval of most medical and veterinary schools. I have discussed this problem with a number of physicians and veterinarians and find that they would appreciate having students who already are familiar with such things as a mosquito, a tick, or a mite. The objection is, that too often there is an attempt on the part of the undergraduate teacher to give an unwanted medical slant to the subject he is teaching. The teacher in the medical and veterinary school prefers to guide his students in the philosophies of his own profession. Such being the case, if undergraduate courses were presented from a zoological point of view, most of the objections to increased entomological training in undergraduate schools could be met.

With our current trend towards greater specialization entomologists will be assuming more significant roles in the fields of medical and veterinary entomology. If we as entomologists are to improve our usefulness in these fields of science, then we must train ourselves better and increase our usefulness in these fields. Likewise, if physicians and veterinarians are to control more effectively insect-borne diseases of man and animals they must be more willing to accept the assistance of the specialist in insect identification, biology, and control. Of course the final answer lies in the close coordination and cooperation of all working harmoniously together as a skilled team.

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# Recent Research on Insects Affecting Man and Livestock

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## ABSTRACT

A new repellent, N,N-diethyl-m-toluamide, was effective against more species than any single compound and superior to most of the mixtures previously tested. It is effective against several species of mosquitoes, deer flies, *Culicoides*, and ticks. Malathion and Chlorthion were effective against human body lice at 0.001 percent in patch tests. Eggs were destroyed when dipped in 0.05 and 0.1 percent acetone solutions of the insecticides, respectively. Malathion, Bayer L 13/59 and Diazinon when used in baits in field tests controlled highly resistant house flies. Malathion applied from aircraft at dosages of 0.1 to 0.5 lb. per acre controlled resistant *Aedes* and *Culex* adults. Solubilized formulations of parathion dripped in ricefield irrigation water controlled *Psorophora* larvae. Some organic phosphorus insecticides show systemic action in animals against screwworms, lice, biting flies, and cattle grubs. Dow ET-57, given orally to cattle, destroyed young grubs in the body before they penetrated the hide. Numerous organic phosphorus compounds tested against cattle and goat lice were effective at low concentrations, but may not be practical because of toxicity. Malathion was practical and effective against fowl mites. Five percent DDT in oil solutions applied from airplanes over slow-moving streams controlled simuliid larvae.

## INTRODUCTION

Some of the recent research conducted by the U.S. Department of Agriculture on insects of medical and veterinary importance will be reviewed in this paper. Most of this research is being done at Orlando, Fla., Kerrville, Tex., and Corvallis, Oreg. The work at Orlando is directed toward the development of new and more effective materials and methods to control arthropods that are of concern to the military services. The findings are also of considerable value to civilian organizations that have an interest in noxious and disease-carrying arthropods.

## INSECT REPELLENTS

In repellent research the greatest advance has been the discovery and extensive entomological evaluation of diethyltoluamide. The technical material contains about 70 percent of the meta, 25 percent of the para, and 5 percent of the ortho isomers, the percentages varying slightly from lot to lot. The synthesis of the compound was reported by McCabe *et al.* (1954). During the last 3 or 4 years I. H. Gilbert, H. K. Gouck, and Carroll N. Smith at the Orlando laboratory have demonstrated that this repellent is effective against more species than any other single compound and superior to most of the mixtures heretofore prepared and tested. The repellent possesses desirable cosmetic characteristics. It is non-oily, dries quickly on the skin to an almost invisible film, and is the choice of most research people for use on the skin. Gilbert and Gouck (1955) and Gilbert, Gouck, and Smith (1955) have evaluated this and other compounds against several species.

Table I shows the comparative effectiveness of this compound and ethyl hexanediol and the mixture M-2020 against several species of mosquitoes and biting flies. Diethyltoluamide is markedly superior to ethyl hexanediol against *Aedes aegypti* (L.), *A. taeniorhynchus* (Wied.) and *sollicitans* (Walk.), and *A. communis* (Deg.) and also more effective against the other species listed.

Four concentrations of ethyl hexanediol and diethyltoluamide were compared in laboratory tests with *Aedes aegypti*. The data in Table II were used in computing concentration-protection time regressions. The concentrations required for 100-percent protection for 200 minutes were 22.4 percent for diethyltoluamide and 52.1 percent for ethyl hexanediol; in other words, diethyltoluamide was about 2.34 times as effective as ethyl hexanediol.

An interesting characteristic of diethyltoluamide is its resistance to rub-off after application to the skin. In order to measure this resistance a standard tissue paper used for wiping was folded, wrapped around the treated arm, and drawn over the treated surface under standardized pressure. Table III shows that this repellent was many times more resistant to wiping than the others. This property is extremely valuable in the practical use of repellents for protection against insects.

TABLE I—Relative Repellency of Diethyltoluamide, Ethyl Hexanediol, and M-2020 as Skin Applications Against Various Species of Mosquitoes and Biting Flies.

Species	Concentration of repellent %	Number of tests	Average protection time (minutes)			Ratio to—	
			Diethyl- toluamide	M-2020*	Ethyl hexanediol	M-2020*	Ethyl hexanediol
<i>Aedes aegypti</i> (L.).	50	144	387	163	—	2.37	—
		61	400	—	152	—	2.63
	100	29	798	362	—	2.20	—
		26	799	—	284	—	2.81
<i>Stomoxys calcitrans</i> (L.)	25	12	480	199	92	2.41	5.22
<i>Aedes taeniorhynchus</i> (Wied.) and <i>sollicitans</i> (Walk.)	50	24	374	177	—	2.11	—
		93	322	—	208	—	1.55
	100	10	578	307	—	1.88	—
		7	563	—	349	—	1.61
<i>Aedes dorsalis</i> (Meig.)	50	4	404	224	304	1.80	1.33
<i>Aedes communis</i> (Deg.)	25	8	386	195	146	1.98	2.64
<i>Chrysops atlantica</i> Pech.	25	5	32	25	—	1.27	—
	100	14	137	90	—	1.52	—
<i>Chrysops discalis</i> Will.	25	4	119	108	106	1.10	1.12
<i>Culicoides canithorax</i> Hoff.	25	7	64	59	55	1.09	1.16
	100	7	576+	469	489	1.23+	1.18+

\*Dimethyl phthalate 40%, ethyl hexanediol 30%, and dimethyl carbate 30%.

TABLE II—Relative Effectiveness Against *Aedes aegypti* of Four Concentrations of Repellents in Ethanol Solutions (14 tests).

Repellent and concentration (%)	Protection time (minutes)	
	Range	Average
Diethyltoluamide		
12.5	38–198	97
25.0	91–367	247
37.5	285–475	356
50.0	320–493	412
Ethyl hexanediol		
12.5	30–105	79
25.0	30–168	80
37.5	72–283	143
50.0	100–286	205

BODY LICE

Research on the body louse (*Pediculus humanus humanus* L.) has been conducted by M. M. Cole and associates at Orlando. Since the body louse became resistant to DDT in Korea (Hurlburt *et al.* 1952, Eddy 1952), efforts have been made to find substitute insecticides. A 1-percent lindane powder developed at Orlando and used by the military services has given excellent protection, but lice may also develop a resistance to this material. Cole and Burden (1956) showed that some of the organic phosphorus insecticides are highly effective at low concentrations, but most of them might not be rated safe for human use because they lower the cholinesterase activity. However, a few of the materials, such as malathion, and Chlorthion, are considered comparatively safe. These materials at 0.1-percent concentration in pyrophyllite were completely effective for 14 days against adult lice exposed to treated cloth in laboratory tests.



TABLE III—Resistance of Repellents to Wiping when used as Skin Applications Against the Yellow-fever and Salt-marsh Mosquitoes.

Repellent	Concentration (%)	Average number of wipes withstood against		Ratio to M-2020	
		Yellow fever	Salt-marsh	Yellow fever	Salt-marsh
Ethyl hexanediol	100	12.9	21.0	1.3	1.5
<i>o</i> -Ethoxy- <i>N,N</i> -diethylbenzamide	100	2.2	25.5	0.2	1.8
Diethyltoluamide	100	42.2	41.0	4.9	3.0
	50	25.3	—	11.6	—
M-2020	100	8.6	13.6	1.0	1.0
	50	2.2	—	1.0	—

The phosphorus insecticides are excellent ovicides, and some of them, such as sulfotepp, caused complete kill of body louse eggs when dipped in acetone solutions at concentrations as low as 0.0000025 percent. Malathion and Chlorthion in acetone solutions destroyed louse eggs when tested at 0.05 and 0.1 percent, respectively. Ovicides prepared as dusts with pyrophyllite were not as effective as solutions. Eggs exposed to dusts containing 1 and 0.5 percent of the ovicide showed 100 and 97.6 percent kill with malathion and 96.7 and 65.9 percent with Chlorthion.

#### HOUSE FLIES

Resistance to DDT and other chlorinated hydrocarbon insecticides has made control of house flies with these materials extremely difficult and in many places impossible. Research by J. B. Gahan, W. C. McDuffie, H. G. Wilson, and J. C. Keller of the Orlando laboratory demonstrated that baits containing organic phosphorus insecticides give excellent control of resistant house flies. Dry sugar containing 0.25 to 1 percent of malathion, Bayer L 13/59, or Diazinon provided good control when sprinkled over barn floors at the rate of about 1 ounce per 1,000 square feet (Gahan *et al.* 1954a, 1954b). Baits containing 2 percent of malathion or 1 percent of Shell OS 2046 in malt or molasses greatly reduced house flies and blow flies over municipal garbage dumps (Keller *et al.* 1956).

Because both liquid and granular scattered baits must be applied every day or two, attempts were made to devise bait stations that would not require attention for several weeks. The bait stations consisted of pieces of screen wire 4 inches square coated with a sweetened paste containing 2 percent of malathion, Bayer L 13/59, or Diazinon. A 6-inch wooden handle was nailed to the wire patches so that the device could be placed upright in the ground or stapled to posts and walls. In most of the tests better than 90 percent fly control was obtained for periods ranging from 40 to 60 days.

There is considerable concern as to whether flies will become resistant to the organic phosphorus insecticides. In order to check on what is occurring in the field, several strains of flies were collected from barnyards near Orlando where these insecticides had been used for several years. The progeny of these flies were found to be 10 to 20 times as resistant as the normal laboratory colony to malathion and L 13/59. The flies in a colony established from one of the field strains were given food containing 0.02 percent of L 13/59 each generation in an attempt to build up resistance, but the resistance did not seem to increase over a 2-year period.

#### COCKROACHES

The resistance of the German cockroach (*Blattella germanica* (L.)) to chlordane and other chlorinated hydrocarbons stimulated attempts to find substitute insecticides. In tests with several hundred chemicals and combinations J. C. Keller, P. H. Clark, and C. S. Lofgren of the Orlando laboratory found some of the organic phosphorus insecticides effective against chlordane-resistant roaches. One of these, malathion, has received USDA label approval for use in homes and other buildings. It is recommended as a 2- to 4-percent preparation for application with a paint brush or coarse spray in roach harborages. Diazinon

has label approval, but its use is restricted to pest-control operators or other qualified personnel.

In practical tests in houses infested with three species not resistant to chlordane—*Periplaneta americana* (L.), *P. australasiae* (F.), and *P. brunnea* Burm.—malathion, Bayer L 13/59, Diazinon, and Am. Cyanamid 4124 gave good protection for 31 to 45 days, and chlordane for 59 days. Oil sprays containing 0.5 percent of dieldrin or 2 percent of chlordane were both fairly effective for as long as 4 months in some tests, but not against chlordane-resistant German roaches.

The effectiveness of poison baits for the control of American cockroaches has been studied at Orlando. Cornmeal, dried ox blood, starch paste, Coca-Cola syrup, root beer syrup, malt extract, dextrin, and honey were the most attractive materials found. Bayer L 13/59, Diazinon, and Shell OS 2046 were the best toxicants. Practical tests in houses indicated that a bait containing 73 percent of cornmeal, 25 percent of Coca-Cola syrup, and 2 percent of Bayer L 13/59 provided good control. From 1/2 to 1 pound of the bait was sprinkled around roach harborages.

Several colonies of German cockroaches collected at various places in the United States were found to be resistant to chlordane, dieldrin, and lindane. One colony from Fort Rucker, Ala., was found to be resistant to pyrethrum. These roaches were approximately 13 times as resistant to pyrethrum and 8 times as resistant to pyrethrum plus piperonyl butoxide as the regular laboratory strain when exposed to sprays. In residue tests the Fort Rucker strain needed an exposure period 31 times that required by regular roaches to obtain a 90-percent mortality.

#### MOSQUITOES

The failure of DDT and other chlorinated hydrocarbon insecticides to control adults of *Culex tarsalis* Coq. in California prompted research by our Corvallis, Oreg., laboratory in cooperation with the Bureau of Vector Control of the California Department of Public Health to develop substitute materials. In laboratory tests malathion, Chlorthion, Bayer L 13/59, parathion, and EPN were found effective against highly resistant *tarsalis* mosquitoes when applied as mist sprays in a 9,400-cubic foot room (Gjullin, 1954). In an airplane spray test over a mile square area in California, Gjullin and Peters (1956) demonstrated that malathion at 0.46 pound per acre reduced *tarsalis* adults by 51 percent and those of *Aedes nigromaculis* (Ludl.) by 97 percent. The low kill of *tarsalis* was due to the adults' habit of resting in protected places such as buildings, under bridges, and in animal burrows.

Airplane spray tests in Florida by J. B. Gahan and associates showed malathion to give quick reduction of salt-marsh mosquitoes. Applied at 0.5, 0.25, and 0.1 pound per acre, this insecticide gave 95 to 99 percent control of adults within 24 hours. Aerial applications of granules containing Bayer 21/199 at 0.05–0.1 pound per acre gave 90 to 99 percent control of resistant salt-marsh mosquito larvae whereas BHC was ineffective at 0.2 pound of gamma per acre.

The dripping of insecticides into water in irrigation canals and ditches for destruction of mosquito larvae in pastures and rice fields has been studied by many investigators. During World War II, DDT emulsions were tested in rice fields, but the method was not practical because the suspended DDT particles settled out before the water covered the fields and the solvents used with DDT volatilized at the nozzle causing stoppage of flow.

Work by Gahan *et al.* (1955a) on water-soluble or solubilized organic phosphorus insecticides showed that Bayer L 13/59 applied at 1 p.p.m. into irrigation canals destroyed *Aedes nigromaculis* (Ludl.) and *A. dorsalis* (Meig.) in pastures. Later work by Gahan *et al.* (1955b) indicated that solubilized parathion at 0.01–0.02 p.p.m. was highly effective against *Psorophora* larvae in rice fields in Arkansas. The formulation contained 4 parts of an emulsifier, Triton X-100, to 1 part of technical parathion. The treatment was still effective after flowing 0.5 to 1.3 miles through a canal and over 400 feet into a rice field. The success of this experiment was due in great part to a simple gravity-flow dispenser which maintains a constant flow of liquid from a 50-gallon drum (Gahan *et al.* 1955).

#### SYSTEMIC INSECTICIDES FOR CONTROL OF LIVESTOCK INSECTS

A new approach to the control of livestock insects is the use of systemic insecticides. These materials are fed or injected into animals to destroy cattle grubs, lice, ticks, and biting

flies. There is a great need for an improved method to control cattle grubs, since the current use of rotenone as a back spray has many disadvantages. A safe systemic insecticide which will destroy the young grubs within the body of the host with a single treatment before the flesh is injured and before the larvae break through the hide would mark a great advance.

A screening program using guinea pigs as the host and screwworms, stable flies, and ticks as the test arthropods has been under way at Kerrville for several years. Several hundred insecticides have been evaluated, and the promising materials tested on cattle harboring natural infestations of cattle grubs and other pests. Dieldrin, aldrin, and lindane were found to destroy grubs encysted in the backs but not the young grubs within the body (McGregor *et al.* 1955). These compounds stored in the fat of the animals in excessive amounts and therefore are not practical for general use.

During the last three years a few organic phosphorus insecticides were found which performed in a way similar to the chlorinated hydrocarbons when administered internally—i.e., destroyed grubs after they had migrated to the back. Diazinon, Bayer L 13/59, and Chlorthion killed grubs that were in the backs of cattle but not those within the host (McGregor and Radeleff 1954). It is interesting that they did not kill small larvae in the host but did destroy the mature larvae encysted in the skin. Young dipterous larvae of most species are many times easier to kill with insecticides than more mature stages when used directly on the insects. Very likely the mode of action and distribution within the host accounts for lack of kill of young larvae. These insecticides have a mammalian toxicity hazard and other disadvantages that will probably prevent their general use.

Studies conducted at Corvallis, by W. E. Robbins, T. L. Hopkins, G. W. Eddy, R. A. Hoffman, and A. R. Roth have contributed to our knowledge of the effectiveness of systemic materials. Recently this group of researchers administered Diazinon labeled with  $P^{32}$  to a cow at the rate of 25 mg. per kilogram of body weight. Radioactivity was detected in trace amounts in urine, feces, blood, and milk shortly after the insecticide was administered. Approximately 74 percent of the radioactive material was accounted for in the urine, but only trace amounts of this appeared to be unchanged Diazinon. Analyses have revealed two major and two minor metabolic products in the urine. Horn flies feeding on the animal were not killed and presumably did not take up measurable amounts of the insecticide. However, the feces were toxic to house fly and horn fly larvae in samples taken 6 to 48 hours after treatment.

Bayer L 13/59 labeled with  $P^{32}$  was used in a similar way on a grub-infested lactating Hereford cow. It was administered orally at a rate of 25 mg./kg. About 65 percent of the compound was accounted for in the urine after 12 hours. Only about 0.3 percent of this was unchanged L 13/59 as determined by paper chromatographic analysis. The milk was found to be slightly radioactive and it did not partition like L 13/59. Bioassay showed that the radioactive material in the milk was nontoxic to house flies feeding on the milk. The grubs (*Hypoderma bovis* (L.)) showed little uptake of radioactivity, and the mortality was less than previously obtained.

Recently a promising systemic compound uncovered in the screening program has been evaluated by the Corvallis and Kerrville personnel. The insecticide, known as Dow ET-57 and in less purified form as ET-14, is 0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate. It destroys the young grubs in the body of cattle before they break through the hide. In June 1955 five cattle at Kerrville were treated orally with Dow ET-57 at a rate of 100 mg./kg. The time of treatment preceded the normal appearance of *Hypoderma lineatum* (De Vill.) by about 2 or 3 months. Weekly examination showed 98 grubs in the control animals but only one each in four of the treated ones. A test at Corvallis late in 1955 in which the treatment preceded the appearance of *lineatum* by about 2 months, 100 mg./kg. prevented grubs from breaking the hide on the back but 50 mg./kg. did not. On an average 13 grubs per animal appeared in the controls, 1.6 in the group receiving the 100-mg. and 9.5 in those given the 50-mg. treatment. These as well as several other tests demonstrate that Dow ET-57 is an exceptionally promising insecticide for use in control of cattle grubs. However, considerable research is necessary before its limitations and advantages will be known.

#### CATTLE GRUB BIOLOGY

Progress on cattle grub biology has not been pronounced, but a few observations are of interest. In the Kerrville area cattle grubs (only *H. lineatum*) normally appear in the backs

of cattle from late summer through December, the main grub season being November and December. In recent years second and third instars have been most abundant during August, September, and October, with a few appearing in early July. These changes are due to meteorological conditions which influence the time of oviposition and length of pupal period. Young grubs are found in the gullets every month of the year.

In Corvallis monthly examination of gullets of slaughtered cattle by A. R. Roth and G. W. Eddy showed peak numbers in October through December and a gradual decline during January and February. From early March through June none were present, but a few appeared in July. The usual incidence of grubs in the backs of cattle in western Oregon is for *lineatum* to appear in January, reach a peak in February, and decline during March, and *bovis* from early April through June.

#### GOAT AND CATTLE LICE

In evaluating new insecticides against goat lice at Kerrville, C. L. Smith, Rowland Richards, and R. C. Bushland found in small-scale tests that malathion at 0.025 percent, Diazinon at 0.01 percent, Bayer 21/199 and EPN at 0.002 percent, and Strobane at 0.1 percent in dips killed all the goat lice (*Bovicola caprae* (Gurlt) and *limbatus* (Gerv.)) within 48 hours. Examination 4 weeks later showed complete absence of lice. These animals were isolated and there was no opportunity for reinfestation.

In large-scale tests with sprays on flocks of goats, Strobane at 0.5 percent compared favorably with DDT, toxaphene, and chlordane, but heptachlor at 0.25 percent and malathion at 0.5 percent were slightly less effective. EPN at 0.03 percent was definitely inferior.

Numerous organic phosphorus compounds were evaluated against the short-nosed cattle louse by the spot treatment method. Malathion, parathion, Am. Cyanamid 4124, Bayer L 13/59, Bayer 21/199, Diazinon, Pirazinon, EPN, and NPD gave good initial kill but were not as long lasting as DDT. Parathion at 0.05 percent and at 0.01 percent prevented reinfestation for 3 weeks, malathion at 0.5 percent and Diazinon at 0.25 percent for 2 weeks, and DDT at 0.5 percent for 4 weeks.

#### POULTRY INSECTS

R. A. Hoffman of our Corvallis laboratory tested several insecticides for control of ectoparasites of poultry. The northern fowl mite (*Bdellonyssus sylviarum* (C. & F.)) on white leghorn hens was controlled in the laboratory by hand-dusting with lindane at 1 percent and lauseto neu, Bayer 21/199, Bayer L 13/59, Am. Cyanamid 4124, Chlorthion, and malathion at 5 percent. DDT was ineffective at 5 percent. Bayer L 13/59, Chlorthion, and malathion also gave good control when a 2.5-percent dust was sprinkled over the backs of the birds.

Dusts containing 0.5 percent or more of malathion gave complete control of the chicken body louse (*Menacanthus stramineus* (Nitz.)) and shaft louse (*Menopon gallinae* (L.)) on birds held in individual cages, but a 0.25-percent dust was less effective.

A 4-percent malathion dust applied with a rotary duster to flocks on wire at the rate of 4 grams per bird gave practical control of the northern fowl mite for at least 30 days. Malathion in an emulsion spray at 0.25–0.5 percent, 25 ml. per bird, gave good control of this mite in field tests. Litter treatments with malathion for mite control were 80 to 100 percent effective initially when 1 pound of a 4-percent dust was applied to floor areas of 40 to 200 square feet. However, its residual effectiveness was erratic, and only the highest rate could be considered as a good one-application treatment.

#### LIVESTOCK-PROTECTANT SPRAYS

Several organic phosphorus compounds were tested for the protection of cattle against mosquitoes and tabanids at the Corvallis laboratory. Malathion, Bayer L 13/59, Chlorthion, and Bayer 21/199 at 1 percent and Diazinon and Pirazinon at 0.25 percent, applied in emulsions at the rate of 1 quart per animal, did not show any appreciable repellency. None of the treatments proved highly toxic to deer flies and horse flies feeding on the animals, but Chlorthion and L 13/59 caused mortality of engorging mosquitoes for 3 days after spraying.



## BLACK FLIES

In cooperation with the South Carolina Agricultural Experiment Station, research has been under way on the biology and control of black flies. In Jasper County turkey growers experience losses due to a leucocytozoon disease transmitted by *Simulium slossonae* (D. & S.) and perhaps other species. Jones and Richey (1956) found seven species of black flies in the county, but only *S. slossonae* and *congrareenarum* (D. & S.) were observed feeding on turkeys. The larvae were attached to aquatic vegetation in the slow-moving streams present in the area. The immature stages could be collected throughout the year.

Control experiments were conducted by J. B. Gahan and D. W. Anthony and associates by applying larvicides in streams to destroy black fly larvae. Five percent of DDT in kerosene was applied by airplane to plots 11 and 4 square miles in size at the rate of 0.033 pound per acre. In the larger plot 99 percent control was achieved for 7 weeks and 68 to 72 percent during the following 2 weeks, but in the small plot the control was not as good. Dieldrin granules applied in the same way at 0.04 pound per acre gave 100 percent control for 2 weeks and 94 to 98 percent during the following 2 weeks.

## CULICOIDES

At Kerrville R. H. Jones and D. E. Hopkins have been studying the biology and control of *Culicoides* implicated in the transmission of bluetongue disease of sheep. Since *C. variipennis* (Coq.) has been reported as the probable vector of the disease, most attention has been given to this species. A laboratory colony has been established and reared through several generations, but the numbers obtained in each generation have not been large. Larvae are reared in water containing cow manure and soil. Adults take blood meals satisfactorily from the inner ears of rabbits. Field observations indicate that the larvae are numerous around overflows of stock water tanks and other standing water, especially where manure is present. The larvae are found in the upper half-inch underneath shallow water along the shore line. Pupae are concentrated in the mud above the water line.

## CONCLUSION

From this review it will be seen that the highlights of the recent research on insects affecting man and livestock are the finding of a repellent that is superior to any now in use and that some organic phosphorus insecticides show excellent promise against both resistant and nonresistant insects, in sprays as well as systemics. Research is continuing, and we anticipate that steady progress will be made in this important field of entomology.

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# Use of Radiation in Insect Control

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## ABSTRACT

Radiation may affect insect populations in three ways: 1. Massive doses sufficient to cause degeneration of cells and loss of function in vital somatic tissues are lethal. The practical application of gamma-rays to destroy insects infesting stored products is now under consideration. This use of radiation is analogous to such an operation as fumigation for insect control. 2. The gonads of adult insects are much more susceptible to radiation than are somatic tissues. Insects are sterilized by doses that do not kill them. The release of sterilized males to compete for mates with males of a natural population is a form of biological control now being tried on screw-worms (*Callitroga hominivorax* (Cqrl.)). 3. Amounts of irradiation less than the sterilizing dose cause recessive lethal mutations which when recombined in succeeding generations are fatal to homozygous individuals. However, natural selection operates to eliminate recessive lethal mutations, and in the meantime heterozygous individuals may display hybrid vigor. Therefore, the introduction of harmful recessive mutations in a population as a form of biological control analogous to disease introduction seems not to have the promise of lethal or sterilizing radiations.

## INTRODUCTION

Ionizing radiations destroy life by causing physical and chemical changes in the cells that they penetrate. The cytoplasm seems fairly resistant to damage, but the nucleus is the part that is affected. In cells not undergoing division the chromatin material is diffused throughout the nucleus and the cells are much more resistant than those which are dividing. During cell division when the chromosomes are well defined, the organism is particularly susceptible.

It is practical for entomologists to treat insects with beta- and gamma-rays. Beta-rays, being negatively charged electrons, have little penetrating power, and so have limited application for insect control. Gamma-rays, being photons without charge, do have great penetrating effects and hence are the radiations most considered for insect control.

## LETHAL RADIATIONS

The damaging effects of gamma-rays are directly proportional to the dose that an organism receives. Large doses cause such cell damage that tissues degenerate and fail to function so that the irradiated organism dies. A dose that is considered large for one organism may be small for another, depending upon its susceptibility.

Plough (1952) reviewed 39 papers comparing radiation effects on insects, mice, and humans. Data taken from his tabulation follow:

Biological effect	Roentgens (r)		
	Drosophila	Mouse	Man
LD-50	30,000	650	400
LD-100	80,000	800	600
Male-sterilizing dose	5,000	500	500

Sullivan and Grosch (1953), in speculating why insects are so much more resistant than mammals, pointed out that insects have no replaceable epithelium, mucous membrane, hematopoiesis, or lymphatic system. The body of the adult insect is composed chiefly of nerve, muscle, and fat, tissues that are also radiation-resistant in mammals, and is covered with nonliving cuticle. About the only tissues in which cell division continues at a rapid rate are the gonads.

Hassett and Jenkins (1952) irradiated six species of stored-product insects with gamma-rays and concluded that heavy infestations in stored products could be quickly killed with a dose of 65,000 r, and that lighter infestations might be controlled by lesser doses which would prevent reproduction.

Brownell *et al.* (1956), summarizing their research on control of infestations in flour, meal, or grain, estimated that on an industrial scale flour could be irradiated with 25,000 rep (roentgen equivalent physical) at a cost of 2 to 4 cents per 100-pound sack.

Gamma-ray irradiation for the control of stored-products insects may hold promise for practical utilization. However, the irradiation causes changes not only in the insects, but also in the irradiated product. Investigators report that treated foods may have off-flavors and that it is necessary to accumulate more research data to establish that the nutritional quality is not impaired. Insect control by irradiation has problems similar to those posed in the development of a new fumigant.

### STERILIZING RADIATIONS

Gross aberrations in the structure and composition of chromosomes of cells of vital tissues are lethal to insects. To produce such changes in somatic tissue large doses are required. Cell division goes on much more rapidly in the gonads; therefore, much smaller doses harm those tissues. Since proper gonadal function is essential to reproduction but not to continued life of the insect, smaller doses of gamma-rays cause sterilization rather than lethal effects. According to Plough (1952), only one-sixteenth of the lethal dose was required to sterilize *Drosophila* males.

Grosch and Sullivan (1953) established even more remarkable differences in resistance between gonadic and somatic cells. They treated *Habrobracon* (= *Bracon hebetor* (Say)) with 180,250 r of X-rays and found that it made the adults sluggish for a brief time but otherwise had no visible effect. The sterilizing dose for those insects was only 5,000 r. They found that over the range of 7,210 to 180,250 r irradiated insects lived significantly longer than the controls. They speculated that the irradiation might have impaired vital functions so that the treated insects lived more slowly and hence longer.

Whiting (1946) performed a novel experiment in androgenesis. She selected *Habrobracon* females that showed certain dominant heritable characteristics, and treated them with 54,000 r of X-rays, a dose sufficient to destroy chromosomes in the nuclei of developing eggs. Then she mated the treated females to normal males bearing recessive genes. Progeny reared from those females turned out to be haploid males bearing the recessive markings of the male parent, giving proof that, genetically speaking, they had no mother, as all inheritance came from the father. This experiment also illustrates that within a single cell, such as an egg, the cytoplasm can be highly resistant while the chromosomes are destroyed.

Howden (1957) found that the dung beetle *Onthophagus texanus* Shaeffer was sterilized by 5,000 r. When larvae were irradiated with 2,000 r they were unable to develop, usually dying as pupae.

Sterilization may also be induced by radiation originating within the body of an insect. Grosch and Sullivan (1952) and Sullivan and Grosch (1953) fed *Habrobracon* radio-phosphoric acid mixed with honey. A concentration containing 100 microcuries per gram of honey was below the sterilizing dose, but wasps that fed on honey containing 250 microcuries per gram were sterilized. The authors theorized that, since phosphorus is one component of cell nuclei, chromosomes were changed by beta-rays originating within the nucleus. It was found that internal beta irradiation at doses above and below the sterilizing amount had no effect on the insects' length of life. The maximum dose was 1445 microcuries of  $P^{32}$  per gram of honey, which the wasps imbibed at the rate of  $0.49 \pm 0.06$  cu. mm. per female.

### STERILIZATION OF SCREW-WORMS

The experiments that led to the eradication of screw-worms (*Callitroga hominivorax* (Cqrl.)) from the island of Curacao have recently been described (Bushland, Knipling, and Lindquist 1956).

Bushland and Hopkins (1951, 1953) found that screw-worm flies could be sterilized with X-rays or gamma-rays. Males irradiated with 5,000 r of X-rays were incapable of reproduction, but females given this dose remained fertile. Pupae that had developed for 5 days at 80° F. and were within 2 days of emergence were more susceptible than the flies. Male pupae were sterilized with 2,500 r and females with 5,000 r. Irradiated flies did not live quite as long as untreated insects, but there was little difference in longevity following treatments within the range 5,000 to 20,000 r. Thus, the lethal dose for male screw-worms is more than eight times and for females more than four times the sterilizing dose.



The mating behavior of the sterilized males was not affected in cage tests with mixed populations of sterile and normal flies. The ratio of fertile to infertile egg masses was about the same as the ratio of normal to sterile males in the mixed population. The females mated only a single time.

The work with screw-worms was undertaken because E. F. Knippling had proposed that release of sterilized males might be used as a form of biological control. Screw-worms did not exist in the southeastern United States until 1933, when they were presumably introduced through importation of infested livestock. Screw-worms invade many of these States in the summer months, but in normal winters survive only in peninsular Florida. The southeastern population of screw-worms is geographically isolated from the one that survives in Texas and Mexico to infest the Middle West each year.

Lindquist (1955) reviewed research which showed that, even where most abundant, screw-worm populations numbered at most only a few hundred insects per square mile, being dependent upon wounds in neglected domestic livestock and wild mammals as the larval habitat.

Screw-worms are obligatory parasites in nature, but in the laboratory they can be reared in large numbers on a medium composed chiefly of ground meat and blood. There is a new generation every 3 to 4 weeks during warm weather, as the eggs require less than 1 day to hatch and larvae complete their growth in 4 to 7 days. The pupal stage lasts 7 days at 80° F. Mating begins when adults are 2 or 3 days old. Females must be about 5 days old to oviposit. They usually die of old age in about 2 weeks.

The biology of screw-worms seems to fit circumstances that Knippling (1955) has propounded for insects being susceptible to eradication through competition with sterilized males:

- "1. A method of mass rearing of the insect must be available.
2. Adequate dispersion of the released sterile males must be obtained.
3. The sterilization procedure must not adversely affect the mating behavior of the males.
4. The female of the insect to be controlled must normally mate only once, or if more frequent matings occur the sperms from gamma-irradiated males must compete with those from fertile males.
5. The population density of the insect must be inherently low or the population must be reduced by other means to a level which will make it economically feasible to release a dominant population of sterile males over an extended period of time."

After preliminary trials in Florida, an eradication experiment (Baumhover *et al.* 1955) was undertaken on the Caribbean island of Curacao. Screw-worms were reared and sterilized in the USDA laboratory at Orlando, Fla., and shipped by air to Curacao, where they were released and the results were observed by USDA entomologists cooperating with representatives of the Veterinary Service, Government of the Netherland Antilles. The insects were sterilized in the cobalt-60 gamma-ray source constructed in the Biology Division, Oak Ridge National Laboratories, for insect treatment (Darden *et al.* 1954).

Results were appraised chiefly on the basis of observations made on egg masses collected from wounded goats held in pens dispersed over the island. Although a dose of 5,000 r is adequate to sterilize both sexes of screw-worms, the released flies were sterilized with 7,500 r. That dose not only sterilizes females but renders them incapable of oviposition. Hence all egg masses collected were evidence of females in the normal "wild" population. Since before the sterile insects were released the egg masses were invariably fertile, observing whether or not egg masses hatched indicated whether the ovipositing female had mated with a fly of the native population or with a released sterile male.

During April and May weather conditions on the island favored fly increase and screw-worms were so abundant that 15 or more egg masses could be collected in a goat pen each week. Releasing 100 sterile flies of each sex per square mile caused about 15 percent of the native flies to lay eggs that failed to hatch. This was insufficient to cause a reduction in the fly population. For 4 weeks beginning July 12, when weather conditions were a little less favorable and the females were depositing about 10 egg masses per pen

per week, a comparison was made of releasing at rates of 100 and 400 sterilized males per square mile. The 100 rate caused 31 percent of the egg masses to be sterile and the 400 rate 49 percent.

The entire island was then treated at the rate of 400 males per square mile per week, sterilized males being released with an equal number of sterilized females from an airplane flying mile-swaths and covering the island twice weekly. The results are shown in Table I.

TABLE I. Numbers of Egg Masses Obtained in 11 Goat Pens Following Release of Sterilized Male Screw-worm Flies over Island of Curacao at Rate of 400 per Square Mile per Week (from Baumhover *et al.* 1955).

Date	Number Fertile	Sterile	
		Number	Percent
August 9-15	15	34	69
16-22	17	38	69
23-29	17	36	68
August 30-September 5	10	37	79
September 6-12	7	42	86
13-19	3	23	88
20-26	0	10	100
September 27-October 3	0	12	100
October 4-10	0	0	—
11-17	0	0	—
18-24	0	0	—
25-31	0	0	—
November 1-7	0	1	100
8-14	0	1	100
15-21	0	0	—

The last fertile egg mass was collected during the week of September 13. During the next 2 weeks 22 sterile egg masses were collected. Then evidence of fly activity ceased except for two sterile egg masses collected on November 4 and 11. By saturating the island with sterile insects eradication was apparently brought about in 4 months (in four generations).

Although formal record-taking on Curacao was terminated on January 6, 1955, 8 weeks after the last evidence of fly activity, B. A. Bitter of the Veterinary Service continues to observe livestock on the island, and he has found no evidence of screw-worms since the last egg mass was collected.

As screw-worms were at least as numerous on Curacao as they are in the most heavily infested areas in Florida, it is believed that the experiment demonstrates the practicability of eradicating screw-worms from the southeastern United States. In preparation for such an eradication campaign, experiments are now being conducted at Orlando to work out efficient and economical means of rearing, sterilizing, and distributing the 50 million insects that will need to be released each week to equal the 400-males-per-square-mile rate that was successful on Curacao.

EFFECTS OF RECESSIVE LETHAL MUTATIONS

In current literature geneticists are expressing concern over a possible disastrous effect of radiation on the human population. There is some argument as to the amount of radiation that constitutes a serious hazard. According to Plough (1952), normal background radiation exposes all life to about 0.1 r per year. This amount of radiation can account for only a small part of the mutations that occur spontaneously. It is believed that a dose of 30 to 80 r may double the human mutation rate.

Since organisms have evolved through natural selection because their characteristics fit them for their environment, it follows that almost any change in the heritable characteristics will be harmful. When dominant lethal mutations occur, they are usually expressed as sterilizing effects. In order for harmful mutations to affect succeeding generations they must be recessive. They are not expressed when heterozygous, but show up in the progeny

when a recombination of genes makes individuals of a new generation homozygous for the harmful character. These recessive harmful mutations are of greatest concern in human populations because of the value we place on human life. In many areas the human population is stable, or nearly so, and the addition of a few harmful mutations to the genetic inheritance might cause a population decline.

Muller (1956) in discussing radiation damage to *Drosophila* said:

"—the effects of the genetic damage are more strongly exerted in the first generation of offspring than in any subsequent generation. They very gradually subside in the course of many generations as the population is purged by the dying out of the unfit."

Wallace (1951) and Wallace and King (1951) irradiated *Drosophila* males with 7,125 r and females with 1,012.5 r of X-rays. In the first generation 18.3 percent of the offspring showed recessive lethal mutations, but natural selection operated so rapidly that within four generations this percentage was reduced to 10.1. The irradiated population seemed to be more vigorous than the control. Mutations that were deleterious when homozygous formed a superior combination when heterozygous. The flies showed the effect of heterosis, or hybrid vigor.

Thus, it seems that the introduction of recessive lethal mutations into an insect population through the release of irradiated individuals will require additional investigation to determine their potential value. However, such releases may fail, for two reasons. One is that natural selection operates rapidly to eliminate harmful mutations. Secondly, the biotic potential of most insects is so great that a population may not decline from the loss of a considerable number of homozygous immature individuals, especially if the heterozygous brothers and sisters display hybrid vigor.

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# Studies on the Inherent Tolerance of *Anopheles quadrimaculatus* Say to DDT

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## ABSTRACT<sup>1</sup>

A technique is described for obtaining rapid and accurate estimates of tolerance to DDT (1:10,000 = 100 p.p.m.) in third- and fourth-stage larvae and in pupae (1 and 24 hours old) of the LTD strain of *Anopheles quadrimaculatus* Say. The method depends upon the use of freshly prepared suspensions, larvae reared under uniform conditions, accurate knowledge of larval sex and of chronological age. The data presented show that stadial age is more important than sexual differences. With this method, 1 hour old fourth-stage larvae are shown to be 4 to 12 times more susceptible to DDT than 24 and 70 hour old fourth instars, respectively. Pupae are shown to be 20 to 60 times more tolerant to DDT than 1 hour old fourth-stage larvae.

<sup>1</sup>Paper published in *Mosquito News* 17 (1): 1-9. 1957.



# Experimentelle Untersuchungen über die Wirkung von Raupenhaaren auf die menschliche Haut

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## ABSTRACT

The hairs of caterpillars of the following species of *Lepidoptera* were rubbed into the skin of a number of persons: *Thaumetopoea pinivora* Tr., *Dasychira pudibunda* L., *Euproctis chrysorrhoea* L., *Lymantria dispar* L., *L. monacha* L., *Gastropacha quercifolia* L., *Dendrolimus pini* L. and *Arctia caja* L. Reactions of the skin were observed: erythema, papulae, small wheals (urticaria), itching, effusion of blood, pigment-formation and desquamation. There were great differences of intensity and duration with different persons. Generally, after repeated inunction at intervals of one or more days, soon a sensibilisation appeared. At different parts of the body the skin proved to be of varying sensitivity. It seems that there are certain relations between the action and the form, thickness, and dryness of the hairs. Some of the hair types had an effect similar to the stings of blood-sucking insects. With others the effect resembled that of sterilized glass-wool fibres. The question is discussed, whether the effect of the hairs is a mechanical or a toxic one.

Die Raupen mancher Saturniden, Melagopygiden und Limacodiden haben echte, z.T. stark wirkende Giftedornen. Die bei meinen Versuchen verwendeten, in Deutschland häufigen und oft als Schädlinge in Massen auftretenden Raupenarten (*Euproctis chrysorrhoea* L., *Thaumetopoea pinivora* Tr., *Dasychira pudibunda* L., *Dendrolimus pini* L., *Arctia caja* L., *Lymantria monacha* L., *L. dispar* L. und *Gastropacha quercifolia* L.) besitzen aber wahrscheinlich keine giftliefernden Zellen an ihrer Basis. Dennoch ruft die stärkere Berührung mit den Haaren dieser Raupen bei den meisten Menschen eine, manchmal recht heftige und langdauernde Dermatitis hervor, nicht selten auch Augen- und Schleimhautentzündungen. Belästigt werden besonders häufig Schmetterlingssammler und solche Personen, die als Gärtner, Waldarbeiter usw. praktische Bekämpfungsmassnahmen durchführen. Oft werden die beobachteten Erkrankungen dem verwendeten Insektizid zur Last gelegt.

Ziel meiner Untersuchungen war es, festzustellen,

1. ob und in welchem Grade die einzelnen Personen auf die Berührung mit den Raupenhaaren verschieden reagieren,
2. ob und wie schnell nach wiederholter Berührung der gleichen Hautstelle oder auch anderer Körperpartien eine Sensibilisierung eintritt und
3. ob eine Ähnlichkeit besteht zwischen der Wirkung von Raupenhaaren und der Wirkung von Stichen blutsaugender Arthropoden.

Zur Verfügung stellten sich 13 Versuchspersonen verschiedenen Alters (zwischen 17 und 52 Jahren), davon 6 männliche und 7 weibliche. Wie ihre Haut auf die Stiche verschiedener blutsaugender Insekten und Zecken reagiert, ist mir aus eigener Anschauung bekannt. Den Versuchspersonen wurden die lufttrockenen Haare der genannten Raupenarten unter mässig starkem Druck in die Haut eingerieben, und zwar in der Regel an einer etwa 7 qcm grossen Stelle an der Beugeseite des Unterarmes in der Nähe des Ellenbogengelenkes.

Unter den Raupenhaaren, die Hautreaktionen auslösten, lassen sich vier verschiedene Typen unterscheiden:

Typus I: Sehr kurze, dünne Härchen an der Basis gleichmässig in eine feine Spitze auslaufend und zum Ende hin mit distal gerichteten Seitendornen besetzt (Abb. 1 u. 2). Man findet sie nur auf engumgrenzten Stellen abdominaler Tergite, den sog. Spiegeln in grosser Zahl sehr dicht zusammengedrängt. Solche Spiegelhaare besitzen u.a. *Euproctis chrysorrhoea*, in ganz ähnlicher Form auch *Porthesia similis* Fuessly und morphologisch etwas abweichend die *Thaumetopoea*-Arten, bei denen die "Spiegel" schon mit blossen Auge leicht zu erkennen sind.

Typus II: Es handelt sich um längere und dickere Haare, die proximal stumpf und distal konisch, oft in einer langen, aber meistens nicht scharfen Spitze enden. Sie tragen

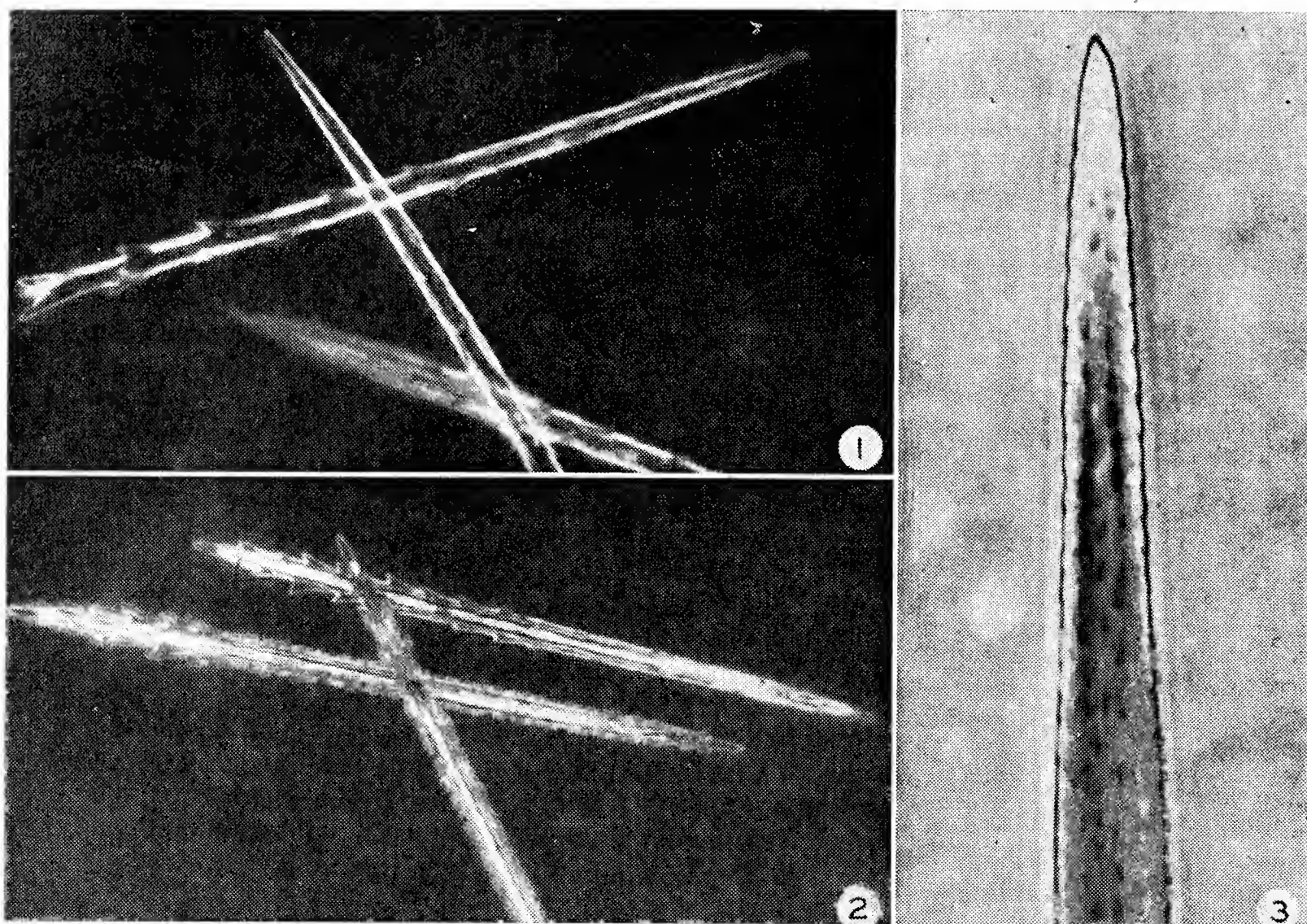


Abb. 1. "Spiegelhaare" von *Euproctis chrysorrhoea*.

Abb. 2. "Spiegelhaare" von *Thaumetopoea pinivora*.

Abb. 3. Spitze eines Haares von *Lasiocampa quercus* (Alle Aufnahmen in starker Vergrößerung mit dem Phasenkontrastmikroskop).

über ihre ganze Länge mit Ausnahme des basalen Teils distal gerichtete Seitendornen, die zur Spitze hin länger, aber spärlicher werden und die beim trockenen Haar leicht abbrechen. Haare dieses Typus haben die Raupen von *Thaumetopoea pinivora*, *Dasychira pudibunda*, *Arctia caja*, *Lymantria monacha*, *L. dispar*, *Acronicta aceris* L. u. a.

Typus III: Die Haare gleichen denen des Typus II, sind aber am basalen Ende nicht wie diese abgestumpft, sondern zugespitzt und fast bis zu dieser Basalspitze mit Seitendornen besetzt. Aus ihnen sind die sog. Bürsten gebildet, die auf abdominalen Tergiten der Raupen von *Dasychira pudibunda*, *Acronicta aceris* u.a. stehen. (vgl. Kemper 1956)

Typus IV: Die Haare sind ziemlich dick, sehr verschieden lang, an der Basis stumpf endend und hier oft mit einer Verdickung versehen. Unter dem Binokular erscheinen sie völlig glatt (ohne Seitendornen). Aber bei starker Mikroskopvergrößerung, besonders bei Verwendung eines Phasenkontrastmikroskops, lassen sich vor dem zugespitzten Distalende sehr feine, oft wie Dachziegel oder Fischschuppen angeordnete, mit dem freien Ende von der Spitze weggerichtete "Widerhäkchen" erkennen. Haare dieser Art fand ich in grosser Zahl bei den Raupen von *Dendrolimus pini*, *Lasiocampa quercus* L., *L. trifolii* Esp. und *Macrothylacia rubi* L. (Abb. 3).

Die Haare aller vier genannten Typen sind von einem Markstrang durchzogen und scheinen in diesem Luft (oder ein anderes Gas) zu enthalten. Ihrer Morphologie entsprechend, können sicherlich die feinen "Spiegelhaare" (Typus I) am leichtesten in die menschliche Haut—mit dem Proximalende voraus—eindringen und sich dort mit Hilfe der Seitendornen verankern. In ähnlicher Weise, der grösseren Dicke wegen aber schwieriger, dürfte das Eindringen auch der Haare des Typus III ("Bürstenhaare") erfolgen. Werden die Haare vom Typus II auf der Haut verrieben, so dringen in diese wohl nur abbrechende Distalenden und Seitendornen ein. In manchen Fällen liess sich das unter dem Mikroskop erkennen. Die dem Typus IV angehörenden Haare dringen mit dem distalen Ende voraus ein, und dann reichen die erwähnten, sehr feinen "Widerhäkchen" wahrscheinlich aus, ein Zurückgleiten zu verhindern.



Die verschiedenen anderen Haarformen, die ausserdem noch bei den oben erwähnten Raupenarten vorkommen—unter ihnen blattförmige, verästelte, spiralig gewundene und sehr biegsame, fadenförmige—spielen für die Dermatitis-erregung wohl keine Rolle, da sie höchstwahrscheinlich nicht in die Haut einzudringen imstande sind.

Als Reaktionen auf das Einreiben der Haare stellte ich fest:

1. den *Einreibeschmerz*. Er wurde in der Regel als sehr schwach, kitzelnd oder prickelnd bezeichnet. Bei Verwendung von Haaren des Typus I war er oft nicht zu spüren.
2. kleine *erythematöse Flecke* von etwa 0,5 mm Durchmesser. Sie traten etwa 5 Minuten nach dem Einreiben wenigstens an dünnhäutigen Körperstellen immer auf und konnten als ein Zeichen dafür bewertet werden, dass Haare oder Haarteilchen in die Haut eingedrungen waren.
3. *Quaddeln*. Sie waren fast immer kleiner und weniger unregelmässig umgrenzt als diejenigen, die in der Regel nach Stichen von Stechmücken, Bettwanzen und anderen Blutsaugern aufzutreten pflegen. Ihr Durchmesser betrug gewöhnlich nur 3—4 mm. Sie verschwanden nach 40—90 Minuten wieder. Manchmal traten sie nach mehreren Stunden (besonders des Abends und am frühen Morgen) spontan für kurze Zeit wieder auf, und es wurde in mehreren Fällen beobachtet, dass sie ein oder zwei Tage nach dem Verschwinden sich erneut wieder einstellten, wenn an einer anderen Körperstelle ein Einreiben mit den Haaren der gleichen Raupenart erfolgt war, dass also hier ein "Repetieren" vorliegt, wie es für die Wirkung von Insektenstichen bekannt ist.
4. *Papeln*, d. s. kleine, meistens stecknadelkopfgrosse, verhärtete Knötchen von mehr oder weniger dunkelroter Färbung. Sie blieben fast immer mehrere Tage, oft wochenlang erkennbar.
5. umfangreiches *Erythem*, verbunden mit lokaler Temperaturerhöhung.
6. *Juckreiz*. Er trat fast immer auf, wenn Quaddel—, Papel- oder Erythembildung erfolgte. Auffällig war, dass er in seiner Intensität viel stärker wechselte, als es nach Insektenstichen der Fall ist. Er wurde niemals lange Zeit hindurch gefühlt, sondern nach mehr oder weniger langen Unterbrechungen immer nur für wenige Minuten. Meistens trat er besonders häufig und stark in den späten Abend- und frühen Morgenstunden (wohl infolge der Bettwärme) auf.
7. *Bluterguss*, d.h. ein Austreten von Blut aus verletzten Gefässen in das Unterhautbindegewebe, erkennbar daran, dass die betreffende Stelle sich zuerst violett-blau und später grünlich und bräunlich verfärbte.
8. *Pigmentierung*, eine leicht bräunliche Verfärbung. Sie trat, nachdem die anderen Hautreaktionen abgeklungen waren, erst nach einigen Wochen und besonders dann auf, wenn ein und dieselbe Hautstelle zu wiederholten Malen mit den Haaren behandelt war.
9. *Abschilferung* (Schuppenbildung), ebenfalls als Spätreaktion und meistens nach wiederholtem Einreiben der Haare an der gleichen Hautstelle.

Nur die beiden erstgenannten Reaktionen, die offenbar rein mechanisch verursacht sind, konnten, wenn auch nicht in allen Fällen, so doch bei allen Versuchspersonen und von allen 4 genannten Raupenhaar-Typen ausgelöst werden. Für die übrigen Reaktionen traf das keineswegs zu. Sie blieben immer bei einem mehr oder weniger grossen Teil der Versuchspersonen nach dem erstmaligen Einreiben aus. Diese anfängliche Reaktionslosigkeit war am häufigsten gegenüber Haaren des Typus I, und zwar beobachteten wir sie gegenüber *Euproctis chrysorrhoea* bei 6 von 13 und gegenüber *Thaumetopoea pinivora* bei 4 von 10 Personen.

Bluterguss, Pigmentierung und Abschilferung wurden in deutlicher Ausprägung nur nach Einreiben der Haare von *Dasychira pudibunda*, *Lymantria monacha* und *Gastropacha quercifolia* (Haartypen III und IV) beobachtet, starkes und umfangreiches Erythem trat nur nach Einreiben von *Euproctis*- und *Thaumetopoea*-Haaren (Typus I) auf. Wenn Quaddeln und Papeln auftraten, gingen die ersteren den letzteren stets zeitlich voraus. Oft stellten sich aber Papeln ein, ohne dass vorher Quaddeln dagewesen wären.

Wie zu erwarten war, zeigten die einzelnen Versuchspersonen hinsichtlich Art, Intensität und Zeitdauer der Reaktionen eine grosse Unterschiedlichkeit. Diese war gegenüber den Haaren von *Dasychira pudibunda* (Typus II und III) am geringsten und gegenüber denen von *Euproctis* und *Thaumetopoea* am grössten (Typus I). Eine der Versuchspersonen muss als überempfindlich bezeichnet werden. Sie reagierte vor allem auf die Haare vom Typus I mit sehr umfangreichem Erythem, heftigem Juckreiz, Quaddeln und Papeln. Auch auf Insektenstiche und einige Medikamente reagiert sie übnormal stark. Andere Versuchspersonen blieben nach dem erstmaligen Einreiben (abgesehen von Einreibeschmerz und erythematösen Flecken) völlig reaktionslos.

Um die Frage der *Sensibilisierung* zu prüfen, wurde bei den zunächst nur schwach oder gar nicht reagierenden Personen die gleiche Hautstelle, meist in Abständen von je einem Tage, wiederholt mit den Haaren eingerieben. Gegenüber den Haaren vom Typus I (*Euproctis* und *Thaumetopoea*) wurde hierdurch, bis auf eine Ausnahme, immer eine eindeutige und starke Sensibilisierung erreicht, und zwar ziemlich schnell, schon nach der 3., 4. und spätestens nach der 5. Behandlung. Die eine (weibliche) Versuchsperson konnte auch durch 15maliges und besonders starkes Einreiben (ausser dem manchmal vorhandenen Einreibeschmerz und den kleinen erythematösen Flecken) durch *Euproctis*-Haare nicht sensibilisiert werden, ebenso wenig auch gegenüber den Stichen von *Aedes aegypti* und *Cimex lectularius*. Auf die Haare von *Thaumetopoea pinivora* und *Dasychira pudibunda* reagierte sie von Anfang an mit Papeln und Juckreiz.

Eine *Desensibilisierung*, ein Unempfindlicherwerden nach wiederholter Applikation, konnten wir weder gegenüber den Raupenhaaren noch gegenüber Insektenstichen beobachten. Wenn durch wiederholtes Einreiben an einer bestimmten Hautstelle, z. B. am linken Unterarm, Sensibilisierung erzeugt war, so konnte diese auch an anderen Körperteilen, z. B. am rechten Unterarm, festgestellt werden.

Die Unterschiede in der Empfindlichkeit der einzelnen Körperstellen gegenüber den Raupenhaaren habe ich in grösserem Umfang bisher nur bei mir selbst untersuchen können. Nach Einreiben von Haaren des Typus I traten keine Reaktionen auf: an der Hand (auch nicht an den Interdigitalhäuten, das sei betont, weil in der Literatur oft die Interdigitalhäute als besonders empfindlich bezeichnet wurden) sowie an den Streckseiten der Arme und Beine. Als wenig empfindlich erwiesen sich die Waden. Normal stark und gleichartig reagierte die Haut an den Beugeseiten der Arme (am distalen Teil der Unterarme schwächer als am proximalen), in den Kniekehlen, in der Leisten- und Nabelgegend und an den Vorderrändern der Achselhöhlen. Abweichend verhielt sich die Haut an Kopf und Hals. Juckreiz und Erythem traten hier schneller und viel stärker auf als anderswo, Quaddel- und Papelbildung aber fehlten. Am empfindlichsten zeigte sich die ventrale Halsseite und das Kinn, weniger stark reagierten die Wangen und am schwächsten Stirn und Nacken. An den Schleimhäuten der Unterlippe rief das Einreiben sofort einen starken, mehr brennenden als juckenden Schmerz hervor, der innerhalb von 1—2 Stunden allmählich verschwand. Sichtbare Veränderungen traten dabei nicht auf. Bei Verwendung der anderen Haartypen waren die Unterschiede der Reaktionen an den einzelnen Hautstellen weniger gross. Mit Haaren vom Typus III und IV liessen sich in den meisten Fällen—vor allem, wenn das Einreiben unter stärkerem Druck erfolgte—auch an den Interdigitalhäuten, den Streckseiten der Arme, der Handoberseite und sogar an der Handinnenfläche eine Reaktion auslösen, allerdings niemals Quaddeln oder Papeln, wohl aber Erythemata, oft starker Juckreiz und kleine Blutergüsse.

Immer noch umstritten ist die Frage, ob die von mir verwendeten Raupenhaare rein mechanisch oder toxisch wirken. Um hierzu einen Beitrag zu liefern, habe ich mit sorgfältig gereinigter und sterilisierter Glaswolle Einreiberversuche wie mit den Raupenhaaren durchgeführt. Die Glaswollefäden, die also nur mechanisch wirken und Hautverletzungen in ähnlicher Grössenordnung wie die dickeren und mit Seitendornen besetzten Haare verursachen können, riefen bei mir und 5 anderen Versuchspersonen niemals Quaddeln und Juckreiz, selten ein geringfügiges Erythem und vereinzelte sehr kleine Papeln, immer einen ziemlich intensiven Einreibeschmerz sowie Bluterguss und nach Wiederholungen Abschilferung hervor. Eine Sensibilisierung konnte hier nicht erreicht werden. Die Wirkung der Glaswolle hat eine gewisse Ähnlichkeit mit der von Raupenhaaren der Typen II, III und IV. Sie unterscheidet sich aber in vielen bedeutungsvollen Punkten (z.B. der Unabhängigkeit von der Konstitution der Versuchsperson und dem Ausbleiben einer Sensibilisierung).

ung) sehr weitgehend von der Wirkung der feinen "Spiegelhaare" (Typus I), die ihrerseits viel Ähnlichkeit aufweisen mit der ja nachweislich toxischen Wirkung der Stiche blut-saugender Arthropoden. Es scheint mir berechtigt zu sein, anzunehmen, dass die Wirkung aller hier in Frage stehender Raupenhaare sowohl eine mechanische wie auch eine toxische Komponente hat und dass die letztgenannte bei den "Spiegelhaaren", die erstgennante aber bei den Haaren der drei anderen Typen vorherrschend ist. Wir brauchen nicht anzunehmen, dass ein Gift, von besonderen Giftdrüsenzellen geliefert, in den Marksträngen vorhanden oder durch Stoffwechselprodukte den Haaren zugefügt wird, denn es ist heute bekannt, dass der sog. Chitinpanzer und damit auch die Haare der Insekten keineswegs nur aus Chitin bestehen, sondern zu etwa einem Drittel aus Proteinen und Lipoiden gebildet werden; und diese sind wahrscheinlich für die toxische Komponente der Wirkung verantwortlich. Sie kommen bei den sehr feinen, besonders leicht und tief in die Haut eindringenden Haaren des Typus I inniger mit dem Blut des Menschen in Berührung und können deshalb stärkere und spezifischere Reaktionen auslösen.

Die in der Literatur mehrfach zu findende Angabe, dass ältere, trockene und deshalb sprödere Raupenhaare stärker wirksam sind, als lebensfrische, feuchte und dann oft biegsame Haare, konnten wir bestätigen. Sie ist wohl so zu erklären, dass die erstgenannten oder abgebrochene Teile von ihnen rein mechanisch leichter in die Haut eingerieben werden können. Unsere Versuche, die Raupenhaar-Wirkung durch Antihistaminpräparate abzuschwächen oder ganz zu unterbinden bzw. zu beheben, hatten bei einigen Versuchspersonen ein positives, bei anderen aber ein nicht eindeutiges Ergebnis.

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### DISCUSSION

C. F. A. BRUIJNING. A species of butterfly from French Guiana has four types of hairs. One of this type causes a heavy erythema when placed on the skin. Months after the butterfly has died the hairs still are very active.

H. KEMPER. Vermutlich handelt es sich dabei um Tiere mit Giftdrüsen oder um Haare, die Gift in Markstrang besitzen.

S. BETTINI. I would like to know what type of antihistaminic substance Dr. Kemper has used and if he has tried cortisone treatment.

H. KEMPER. Verwendet wurde Solventol. Mit Cortison haben wir keine grösseren Erfahrungen.





# Recent Cattle Grub Life-History Studies at Kamloops, British Columbia, and Lethbridge, Alberta<sup>1</sup>

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## ABSTRACT

Methods of girdling cattle at Kamloops to collect and rear large numbers of warble grubs are described. Commencement of *H. lineatum* grub emergence, 1948 to 1952, varied between Feb. 12 and Mar. 10; the period of 50% emergence varied between Mar. 14 and 31. Daily peak of emergence of grubs from cattle occurred about noon. The threshold temperature for pupation appeared to be 55°F. Fly emergence occurred in mid-May, usually between 7:30 and 8:30 a.m. Of a total of 511 fly releases near confined cattle, none was captured beyond 330 yd.; the greatest recapture, involving 26% of all females released was at 500 ft. Mating occurred within an hour of release. No males were recaptured. Subsequent studies at Lethbridge involved an open-back grub-collecting girdle. Observations during the hypodermal stages of *H. lineatum* and *H. bovis* were charted. The mortality of grubs in the backs of cattle was 59 and 51% for two years. A constant temperature of 20°C. and any R. H. short of 100% for *lineatum*, and 76% for *bovis*, was optimum for pupal development, although unknown lethal factors were still present in lab. rearings. Emergence of flies from *lineatum* larvae dropped in the field was 8 to 80% depending on location. Maximum time from larva to fly emergence was 65 days; minimum 25 days. This period decreased with the progression of the season. A loss of weight during pupation is correlated with cold hardiness and the prepupal moult.

## INTRODUCTION

Studies on the biology of warble grubs (*Hypoderma lineatum* De Villers and *H. bovis* De Geer) and observations on their migration through host tissues from their point of entry received a great impetus during the years 1914 to 1926. Notable workers of this era include Hadwen, Warburton, Fulton, Bishopp, and Laake. During the next few years little was done in Canada to add to basic knowledge of these pests; studies were confined to experiments on control at Kamloops by Eric Hearle and locally in Ontario and Quebec (Gibson and Twinn, 1936). During the war years further life-history studies were deemed advisable and, in 1943, the author and G. P. Holland were assigned projects on the natural flight ranges of adults and the seasonal history of immature stages at Kamloops; their work ceased in 1948 when Holland was transferred to Ottawa. Subsequently, G. B. Rich and J. Weintraub of the Kamloops laboratory studied the survival of the immature forms and experimented with factors influencing flight and oviposition of the flies.

In 1949 studies on the biology of the larvae and pupae were initiated at Lethbridge by J. McLintock. These were suspended when he moved to Ottawa in 1952. The program at both laboratories was reorganized in 1953 when J. Weintraub was transferred to Lethbridge, taking with him the main responsibility of warble studies in Western Canada and leaving G. B. Rich in charge of long-term population studies at Kamloops. It is the purpose of this paper to draw together and summarize the results of the studies of Holland, Gregson, and McLintock.

## STUDIES AT KAMLOOPS

In seeking a method of collecting large numbers of grubs for rearing flies for flight studies at Kamloops, B.C., it was discovered early that viable material could not be collected by hand extraction from hosts. An apparatus consisting of canvas pouches, applied to the sides of infested cattle was made and described (Gregson and Holland, 1944). This was later modified to consist of a large canvas sleeve that fitted entirely around the body of the animal, laced along the top by means of a leather thong through eyelets. When open, the dimensions of the sheet were 28" x 66" for small yearlings and 30" x 70" for larger animals of the same age. Attached below the eyelets of one side was an inner 6" flap, which, in position, lapped under the opposing side. The fore and rear margins of the girdle contained a draw cord by which its ends were tightened against the animal. Attached to the underside of the sleeve was a glass receptacle, screwed into a funnel-like metal collar. The latter consisted of two metal

<sup>1</sup>Contribution No. 3565, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

discs slightly conical in shape, each with a one-inch hole at the center. To the lower was reamed and securely soldered the metal lid of a 2" x 2" wide-mouth jar. A similar hole was cut through the sleeve at a point about 10" behind the lower front margin. The canvas was then bolted between the two discs so that as grubs emerged from the cow they would roll from the sides of the animal through the funnel into the jar. These girdles were reasonably efficient, and collected as many as 139 mature and uninjured grubs from one cow. Because, at the beginning of these experiments, large numbers of grubs were required, the cattle to be girdled were selected for maximum infestations and borrowed for two months from neighbouring ranches. The grubs were usually collected from the girdles twice daily and placed in ground level rearing sites screened against mice.

Information from records of these collections showed no correlation between warmth of day and the number of grubs emerging from their hosts during a given interval. The peak of emergence of 60, 50, 32, 29, 34, and 17 grubs collected March 18–23, 1946, occurred about midday, although the maximum heat of day usually did not occur until about 4 p.m. Few or no grubs emerged at night, and few during afternoons and evenings. Observations made during varying turning out and feeding times of infested cattle were not conclusive, but suggested that the emergence of grubs from the backs was stimulated by activity of the host.

Records of grub emergence at Kamloops from 1948 to 1952 showed that commencement of emergence of *H. lineatum* varied from Feb. 12 in 1948, to Mar. 10 in 1949. The time at which 50 per cent of the larvae had emerged varied from March 14 in 1948, to March 31 in 1949. The longest period of grub emergence was from Feb. 12 to April 12 in 1948, the shortest being from March 3 to April 2 in 1952, periods of 60 and 30 days respectively (Fig. 1).

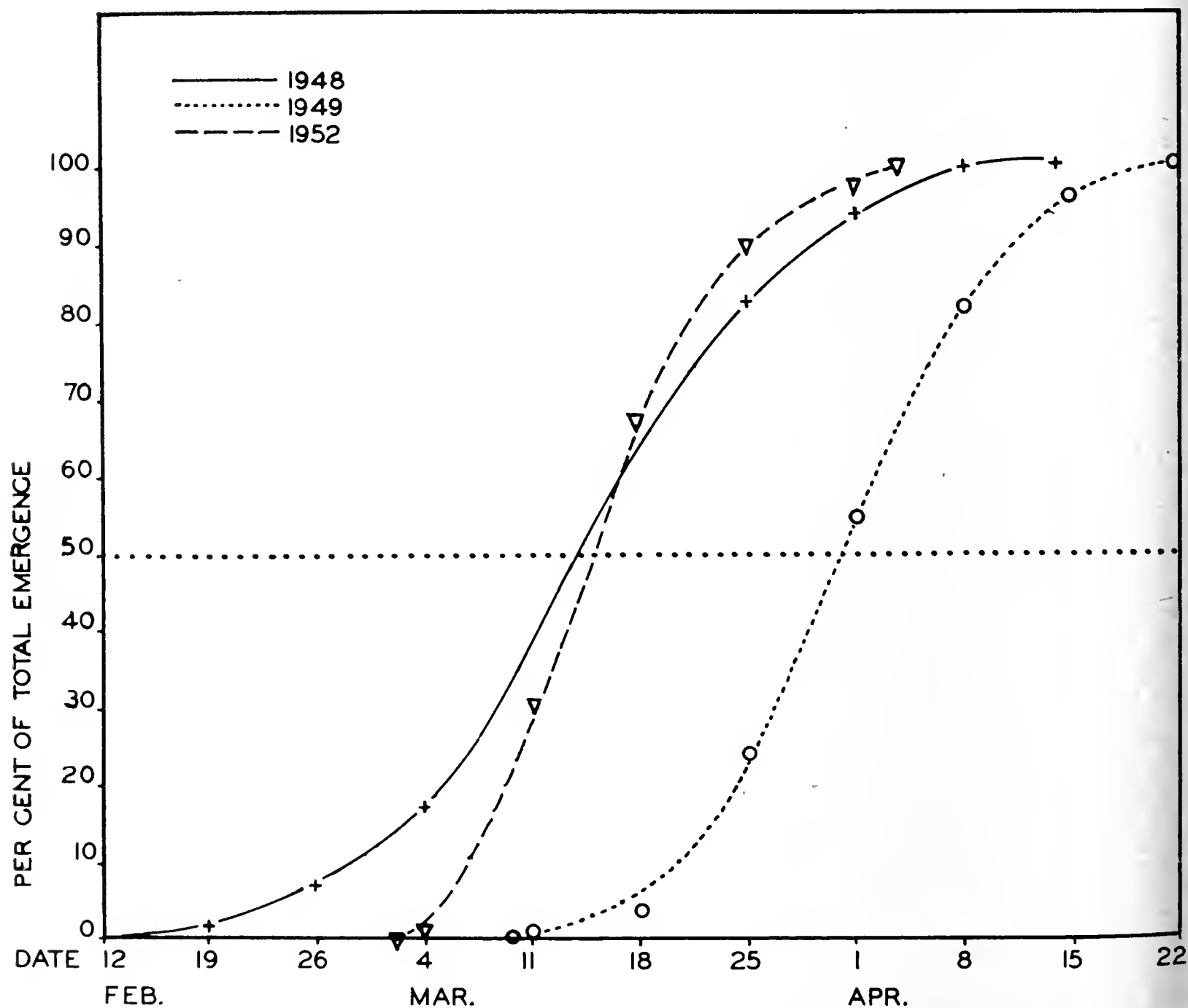


Fig. 1. Variation in times of emergence of *Hypoderma lineatum* larvae from cattle. Kamloops, B.C., 1948, 1949, 1952.

After emerging from its host the grub passes through a prepupal period during which, providing it is warm enough, it moves around actively. Only *H. bovis* was observed to burrow beneath loose soil. The duration of this period varies considerably, the average being about three days. A temperature of 55° F. appears necessary for pupation, but whereas longer prepupal periods occurred during earlier cold weather, later warm days did not bring about consistently shorter intervals.

After pupation there is a quiescent puparial period. This was observed to last 40 to 68 days, depending upon the situation of the puparium and weather conditions. Grubs which pupated in mid March could be expected to produce flies in mid May. However, the puparial period decreases as the date of pupation advanced, thus tending to bring about a concentrated period of fly emergence that is of shorter duration than that occupied by pupation. In nature a greater spread and variation of the period of emergence is caused by the diversity of climatic conditions encountered by the randomly dropped grubs. Such was shown by a series of observations on 17 grubs and 8 puparia placed on each of the following sites March 20–24, 1947:

- Cage 1. Hard bare ground. No protection from the sun. Temperature extremes ranging from 23° F. to 104° F. Flies emerged from April 26 to May 15. Total emergence of 18 flies (72%) included 13 ♂, 5 ♀.
- Cage 2. Loose sand. No vegetation. Complete shade. Temperature extremes 26° F. to 88° F. Flies emerged from May 27 to June 6. Total emergence of 21 flies (84%) included 13 ♂, 8 ♀.
- Cage 3. Manure pile. Broken sun and shade. Grubs 1 to 2 in. below the surface. Temperature extremes 32° F. to 91° F. No emergence. Most remaining puparia showed flies in various stages of development. A few were eaten by insect predators.
- Cage 4. Bare ground with grass stubble. Intermittent sun. Temperature extremes 23° F. to 89° F. Flies emerged from May 27 to June 6. Total emergence of 17 flies (68%) included 11 ♂ and 6 ♀.
- Cage 5. As cage 4, but with loose cover of leaves over grubs. Temperature extremes 23° F. to 89° F. Flies emerged from May 30 to June 4. Total emergence of 11 flies (44%) 6 of which were eaten by ants.

Thus cage 1, in direct sun, produced flies first, whereas cage 2, in complete shade, though later, produced the greatest percentage. The pupation periods in these two locations ranged from 33 to 77 days. That cage 3 produced no flies was not unexpected. Apparently the heat and/or chemical action generated by the manure was lethal to the grubs. Of the unhatched pupae from the five plots examined the majority contained well formed dead flies. More males than females emerged.

Further observations were made in March 1948 on the part played by heat. Two lots of 30 active grubs were selected and placed in sunny and shady cages which had a difference of about 10 degrees in air and soil temperatures. By the third day 25 of the 30 had pupated in the sunny cage, and only 8 in the shade.

Since the threshold of grub activity is about 50° F., it stands to reason that if grubs, due to their location, fail to receive this temperature, pupation may be delayed indefinitely. This was apparently true of grubs that were placed in a cellar, where the maximum temperature remained between 50 to 57° F. Similarly, puparia kept under these conditions failed to develop to flies.

Although prolonged retardation of development is fatal, mild suppressions due to cool weather or shade are not detrimental as evidenced in cage 2 (84% emergence).

Given fairly uniform conditions in which to pass the puparial period, the emergence of warbles as adults, under Kamloops conditions, occurred in mid-May over an interval of little more than two weeks. Daily hatching occurred mainly between the hours of 7:30 and 8:30 a.m. (P.S.T.) whether the air temperature was as low as 47° F. or as high as 55° F. Warmer temperatures or sunshine after these hours failed to bring forth additional flies, and only on rare occasions did a fly emerge after noon. However, laboratory observations were made on a series of mature puparia which had been kept too cool to hatch. One-half of the collection was exposed at 10 a.m. to the sun at a temperature of 104° F. The remainder were placed in a dark incubator at the same temperature. An hour later 5 flies emerged from

puparia in the sun and 9 from those in the incubator, indicating that warmth is the stimulating factor in emergence. The fact that no emergences occurred in the afternoon during field observations is unexplained. Possibly the day's warmth must be preceded immediately by the cooler night. It seems that if a morning is too cool to produce emergence, then no flies appear until next day, regardless of how warm the afternoon becomes.

Males and females emerged at about the same time, both during the daily and seasonal hatchings, there being no noticeable earlier appearance of the males, as in many other insects.

The emergence of each adult fly was heralded by a faint, but audible pop, as the circular operculum of the puparium burst open. Immediately after this, the pulsating balloon-like ptilinum appeared at the opening, and within about 10 seconds the fly levered itself out.

Once free of the puparia, the flies were quiescent for about 10 minutes, during which time the wings were expanding to their final size. At this time drops of meconium fluid were liberated. Once the wings were normal, the flies usually attempted short flights, particularly if exposed to sunshine, though nearly an hour elapsed before all the meconium was eliminated and each was in a condition for normal flight. By then the ptilinum had completely retracted and the swollen abdomen had attained its normal size.

The first flies to leave the experimental plots usually took flight about 9 a.m. (P.T.S.); the rest within the hour. In field observations it has been noted that the flies apparently do not bother cattle until about 10 a.m.

Mating occasionally occurred within an hour of emerging, apparently without any nuptial flight. The process was completed in about two minutes. Positive heliotropism was marked, and mating took place only in direct sunlight.

The life of the adult fly appears to be short. Flies left in flower pots under rearing conditions lived for three to five days, whereas flies kept in the dark in a cellar (temp. 55° F.) lived 10 to 15 days.

Being without mouths they are unable to obtain nourishment and live entirely upon reserves stored in their bodies. Because of this, it may be assumed that aging flies become progressively weaker, and that flies which have been obliged to "sit out" inclement weather probably exhibit less striking powers than newly emerged specimens released under ideal conditions.

The main purpose of the Kamloops studies was to obtain information on the flight range of the warble fly. Experiments were carried out during three seasons on the Lac du Bois range, about two miles from the nearest farm and outside cattle.

The puparia were carried with as little disturbance as possible and placed under screened covers within the sunken sides of 2' x 3' cages. The outer sides of these were encircled by a protected layer of tangle foot to exclude ants.

Observations were made daily, commencing not later than 6 a.m. (P.S.T.) and often as early as sunrise. As the flies emerged they were sexed, recorded and marked with a light touch of diluted Duco colour on the tarsi of one leg. Their activities and subsequent releases or departures were recorded and correlated with temperature and humidity readings. Before their flight observers were stationed by the laboratory cattle to capture and record any flies attracted to them. Unless unfavourable weather set in earlier, these observations were usually continued until about 4 p.m.

During observations in 1946 four cattle were used for bait, three being tethered equidistant, each one-eighth mile from the fourth animal at the centre. The potential fly stock consisted of 400 puparia. As the flies emerged they were marked, grouped, and allowed to mate, after which a third of each day's emergence was liberated at each of three sites equidistant from two adjacent cows and one-sixteenth of a mile from the center and nearest cow. Of 148 flies liberated 11 were recaptured, the farthest distance from the point of liberation being 330 yards. This distance was covered in a minimum period of 95 minutes. In no instance was a fly released the previous day captured.

Having shown that the flies could find cattle over a distance of one-eighth mile, further observations were made in 1947. Four cattle were permitted to move freely in a small corral. The flies were liberated from three positions 350 ft. away and four opposing sites one-fourth mile away. No recoveries were made of the 49 male and 41 female flies released from the quarter-mile points; 3 females were recovered from the 94 males and



88 females released from the 350 ft. point. Two of the recovered flies were one day old. One fly arrived at a cow 21 minutes after release, when it was not more than  $2\frac{1}{2}$  hours old.

Because the proportion of flies recaptured during the two previous year's releases was so low, great care was taken in 1948 to avoid injuring them before their release. Emerging flies were given adequate room for drying, then, without being touched by forceps, were painted on a convenient tarsus and allowed to fly. Stress was thus given to ascertaining the proportion of flies reared under optimum conditions that were capable of finding cattle, without regard to maximum distances of flight. Four cattle were tethered in opposite directions, each 500 ft. from the central release point. Of a total of 191 released, of which 107 were females, 28 females (or 26 per cent of females) were recovered. No males were captured after release. Females ovipositing on the cattle within an hour of release were assumed to have mated during this interval. Most of the flies captured were of the same morning's emergence, although a few instances of one- and two-day old flies attacking cattle were observed.

Flight studies subsequently carried out at Kamloops by G. B. Rich and J. Weintraub (unpublished) showed that *H. lineatum* is capable of flying distances of up to 10 miles on a flight mill. Rich also demonstrated that flight of *H. lineatum* was stimulated by an alternation of light intensities. Atmospheric pressure had no effect on flight velocity.

### STUDIES AT LETHBRIDGE

Research on warble flies was commenced at the Livestock Insect Laboratory, Lethbridge, Alta., in 1949, by J. McLintock. Although this work was designed to elucidate other aspects of the problem than those studied at Kamloops, many of the observations corroborated the earlier studies. Both species of *Hypoderma* were found to extend across southern Alberta from the British Columbia boundary in the Crows Nest Pass to Robsart in southwestern Saskatchewan. Northwards, both species were traced in Alberta to about the 52nd parallel. The highest altitude at which both species were collected was between 4000 and 5000 feet. In Alberta, no cattle-raising locality was found where warble flies were absent.

Girdles were used on cattle to collect live grubs. Because the presence of canvas over the back of an animal might raise the skin temperature and thereby effect the normal emergence of the grubs the girdles were modified to an open-back type. This design involved a strip of canvas 26 to 29 inches wide and 48 to 55 inches long, which hung under the belly of the heifer and was supported at its narrow ends on a harness of cotton webbing and wood. The latter consisted of two pieces of modified 2 x 4 inch hardwood which, when the harness was in place on the heifer, rested along each side of the body eight to 12 inches below the top line.

At the front the boards were suspended by double straps of 4-inch cotton webbing arranged in such a way as to keep them in a vertical position parallel to the body of the heifer. At the back, the boards were supported by a single strap of 4-inch webbing attached in the same manner as the inner strap at the front. The harness was secured to the heifer by straps of one-inch webbing that passed under the belly of the heifer. When the grubs emerged they fell between the boards and the hide of the heifer, thence down the canvas apron and into a pouch.

Of 386 grubs collected with this girdle in the spring of 1952, only 20, or about 5 per cent, were squashed in the girdles. This usually occurred when the canvas apron was too tight around the belly of the heifer. This model was subsequently modified at Kamloops by replacing the wooden supporting slats by curved laths of fiberglass. These proved to be more resilient and better able to withstand rough treatment by range cattle.

The open-back girdles allowed more accurate observations to be made on the length of time that larvae spend in the backs of cattle. Such observations were made at Lethbridge in early January 1951 and 1952. The hair at the sites of punctures in the backs of range cattle was clipped and the positions recorded on charts. Very heavily infested animals were excluded as the punctures were too close together. With the aid of a strong light it was possible to identify the larvae to species *in situ* once they had reached the third instar.

Table I shows the developmental periods in the backs of cattle of the larvae of both species in two years. Figs. 2 and 3 show graphically the duration of larvae that emerged successfully from the backs of sixteen heifers.

TABLE I—Developmental Periods of Warble Larvae in the Backs of 16 Cattle in 1951 and 1952, Lethbridge, Alta.

H. lineatum						H. bovis			
Year	No. of cattle	No. of specimens	No. of days in the back			No. of specimens	No. of days in the back		
			Min.	Max.	Aver.		Min.	Max.	Aver.
1951	5	16	44	63	55	12	60	81	71
1952	11	40	37	65	53	44	44	80	64

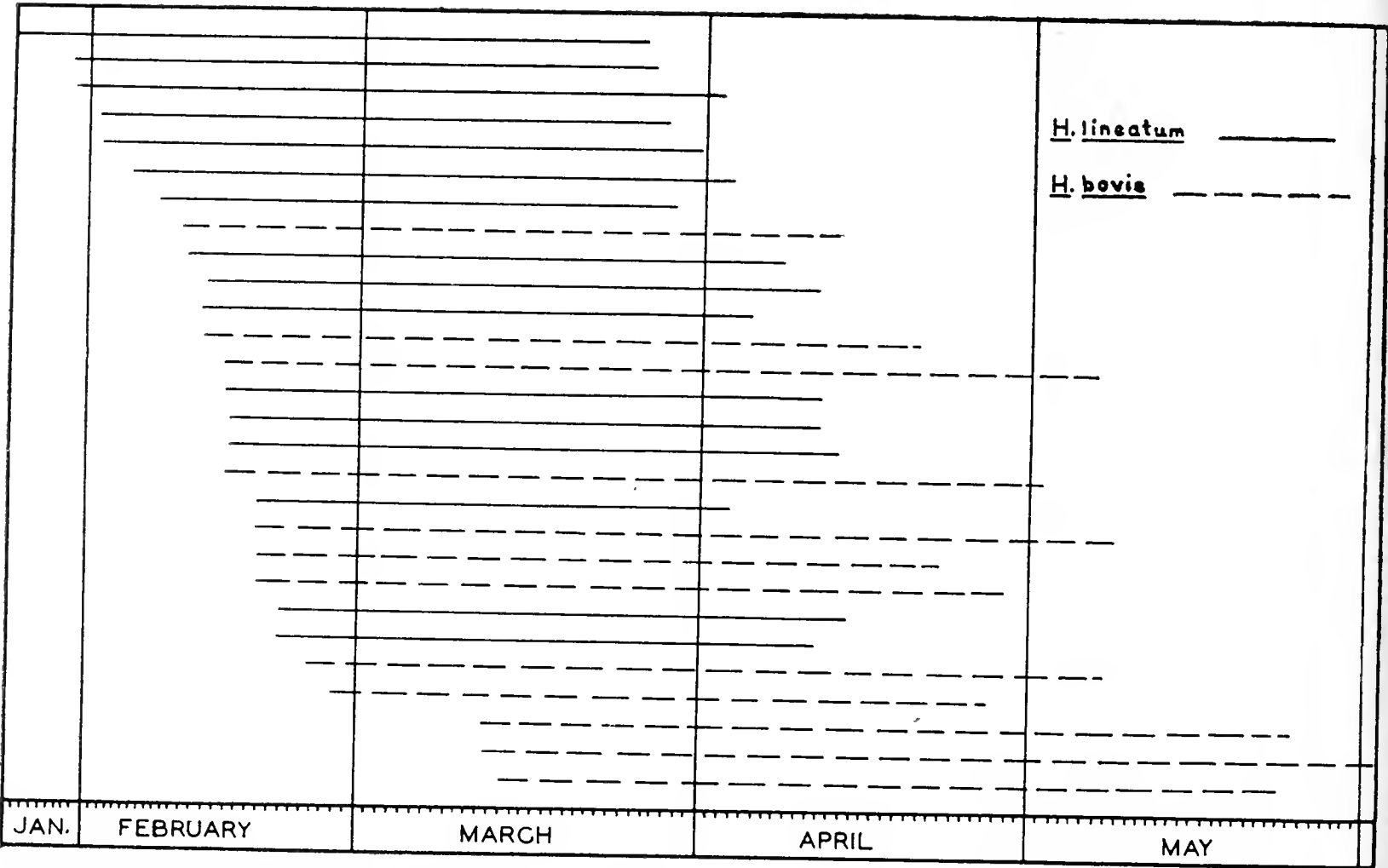


Fig. 2. Duration of *Hypoderma* larvae in backs of five cattle, Lethbridge, Alta. 1951. Solid lines *H. lineatum*; broken lines, *H. bovis*.

The five heifers used in 1951 were each from a different locality. In Fig. 3 the fifteen heifers used are grouped according to their eleven localities. In both figures, only those punctures are included from which normal larvae emerged. The data from the Strathmore and Lousana heifers show an overlap in the occurrence of the two species in the backs of the cattle. This overlap is important in control operations. The 1951 data for the five heifers from different localities indicated that maximum control could be obtained by applying the first treatment about 40 days after the appearance of the first puncture, and the second treatment 28 to 30 days later. If this formula had been followed at Strathmore in 1952, the spraying dates would have been around February 18 and March 18; this would have given optimum control of *lineatum*, but some *bovis* would have been missed. On the other hand, at Lousana the spraying dates would have been around March 5 and April 5, and the two treatments would probably have given optimum control of both species. Attempts to establish a formula of this type for two treatments are made difficult by *H. bovis* which, as in 1952 at Olds and Strathmore, punctures the hide as late as the third week in April. Where both species occur in a herd, the grubs may be present in the backs of the cattle for as long as 146 days. Nevertheless, a study of this type in a herd in a specific locality probably could lead to a reliable spray calendar for that locality. The fact that the later-appearing larvae of both species spend a shorter time in the back suggests that temperature or some other meteorological factor might be employed, along with the data on punctures, to estimate the time of the second treatment.

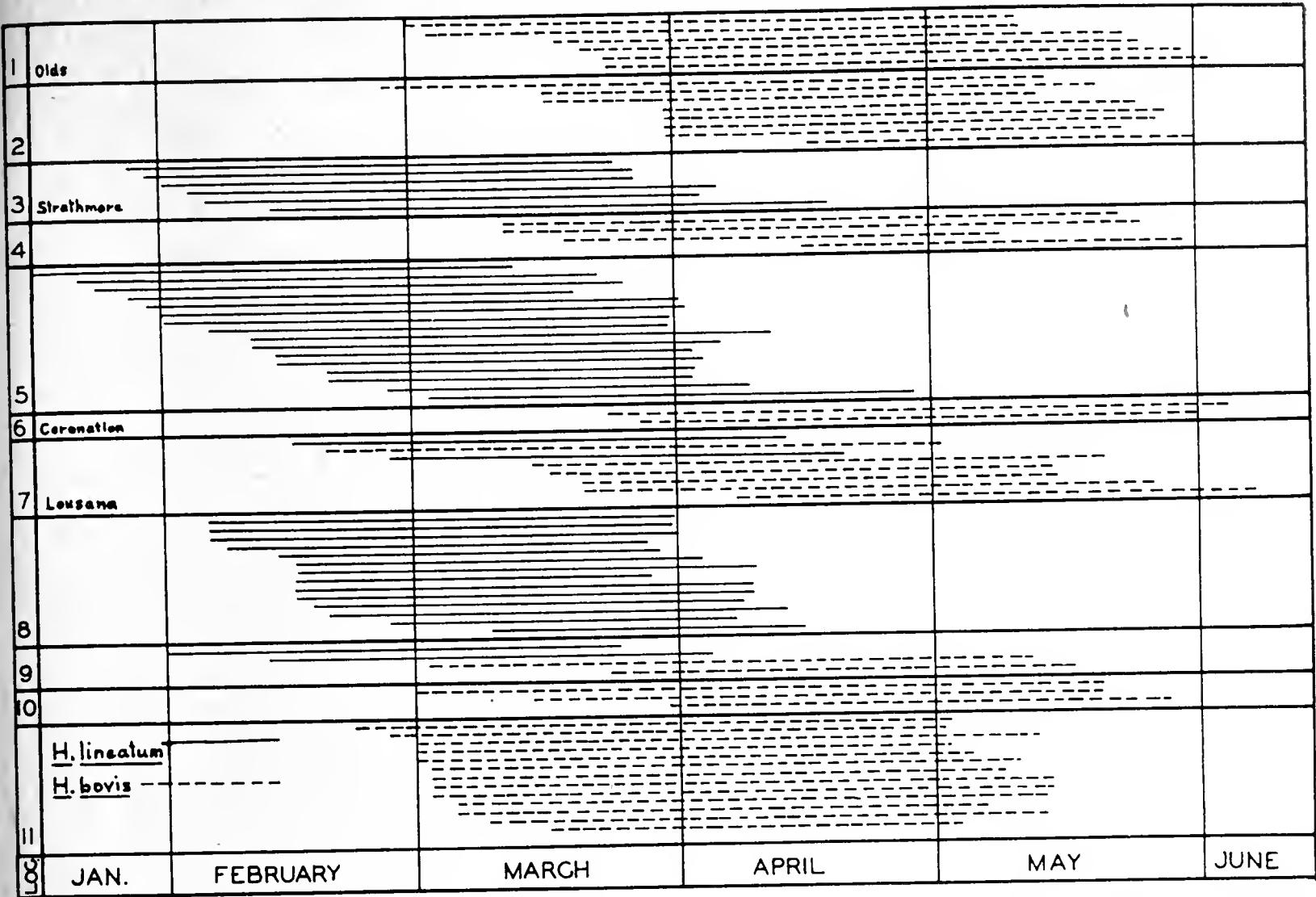


Fig. 3. Period of individual larvae of *Hypoderma* in backs of 15 cattle from 11 localities, Alberta, 1952. Solid lines, *H. lineatum*; broken lines, *H. bovis*.

When observing the punctures in the backs of these cattle it was possible to ascertain the mortality of the hypodermal stage. The average mortalities in the two years were 59 and 51 per cent for totals of 87 and 133 grubs, respectively.

Observations on a portion of the emerged grubs showed that at 60% R.H. the optimum temperature for fly emergence was 20° C. Yet this only gave 61 per cent emergence, so tests were made at 20° C. using constant relative humidities ranging from 0 to 100 per cent. High emergences for *H. lineatum* pupae were obtained at relative humidities from 0 to 98 per cent. These results are in general agreement with those obtained by Pfadt (1947) who concluded that there was very little difference in the survival rate of pupae reared at relative humidities from 0 to 76 per cent. The optimum relative humidity for *H. bovis* pupae appeared to be about 76 per cent both for per cent emergence and duration of the pupal stage.

A great variation in the per cent emergence of *H. lineatum* was observed. This, and the comparatively low per cent emergence of *H. bovis* under apparently optimum conditions of temperature and humidity, indicated that some still unknown lethal factor or factors were operating.

Because of the expense involved in gathering range cattle for warble-fly treatment, the time of application of the first, and sometimes only, treatment is extremely important to the rancher. On the prairies of Western Canada the larvae of *H. lineatum* begin to emerge from the backs of the cattle around the end of February or early in March. These early grubs are usually subjected to comparatively low temperatures at some time during their development after they leave the host. Hence, it is important to know whether or not these early grubs can survive cold weather after they drop to the ground.

Experiments by Salt (1944) at Lethbridge, by Bishopp *et al.* (1926), and by Pfadt (1947) showed undercooling points for larvae and pupae of *lineatum* of from -10° C. to -25° C. Their results were inconclusive and further studies might reveal changes in the cold hardiness of the grub during its development in the puparium.

Observations to determine the development of *H. lineatum* under field conditions were made with 243 larvae. The history of all but 27 that were accidentally plowed under, and 5 that were lost to predators, is summarized in table 2. It will be seen that as the season progressed the average time from dropping of the larvae to emergence of the flies decreased

from 59 to 34 days. The maximum time to emergence was 65 days and the minimum 25 days. In general, this agrees with the information given in the literature for other parts of North America. In Wyoming, Pfadt (1947) found the times to range from 34 to 80 days; Bishopp *et al.* (1926) 30 to 60 days; Bishopp *et al.* (1944) 18 to 77 days.

TABLE II—Emergence of Flies from Pupae of *Hypoderma lineatum* under Field Conditions. Lethbridge, Alta., 1949.

Date larvae dropped	Approx. min. air temp. to which exposed °C.	Number of larvae dropped	Flies emerged	Per cent emergence	Average time to emergence Days
March 2-13	-29	14	6	43	59
March 14	-25	4	2	50	53
March 15-16	-16	12	1	8	53
March 18-25	-10	89	54	61	44
March 27	-8	16	12	75	42
March 29-April 7	-6	76	61	80	34

Pfadt (*loc. cit.*) obtained an average emergence of 95 per cent from 88 specimens under field conditions. The average emergence at Lethbridge was only 53 per cent. Even if the 8 per cent emergence of flies from larvae dropped on March 15-16 is discounted, the average emergence is only 62 per cent. Pfadt's larvae were subject to an average minimum temperature of  $-8^{\circ}\text{C}$ . whereas those at Lethbridge were probably subjected to considerably lower temperatures (a probable average minimum of about  $-15^{\circ}\text{C}$ .) which may account in part for the difference in the two emergence results.

The 12 larvae dropped on March 15-16 (Table II) were in the first two of five lots dropped in a sheltered location in a feed lot. This location was beside a fence and facing south so that it received the sun for most of the day; the ground was well covered with straw, hay, and weeds. Of the 31 larvae placed in this location at different times, only eight, or 26 per cent, produced flies; the remainder either died at the onset of pupation, or the flies were unable to push off the operculum of the puparium.

From 28 larvae dropped in snow, or later covered by snow, 13 flies emerged, or 46 per cent. These specimens were exposed to minimum temperatures ranging from approximately  $-21^{\circ}\text{F}$ . to  $14^{\circ}\text{F}$ . Of those exposed to  $-21^{\circ}\text{F}$ . six flies were obtained from 14 larvae; of those exposed to  $14^{\circ}\text{F}$ . five flies were obtained from 10 larvae. These temperatures are only rough approximations; the temperature under the snow may have been much higher than that of the air when the latter was at its minimum.

Weight changes in puparia during development were often indicative of the metabolic and growth changes taking place. Eighteen puparia maintained at  $20^{\circ}\text{C}$ . and approximately 60 per cent R. H. were weighed daily. The results are summarized in Fig. 4. Between the second and fourth days there was a sudden increase in loss of weight followed by an equally sudden decrease. The daily weight loss then continued to decline slowly until five days before emergence, when it increased slowly until emergence.

That the loss in weight during pupal development is most likely caused by loss of water was indicated by the fact that the percentage of total body weight that is water gradually decreased as the pupae matured. A series of 91 grubs maintained at  $20^{\circ}\text{C}$ . and 60 per cent R. H., and ranging in age<sup>2</sup> from a larva 40 minutes old to a puparium 16-17 days old in which the fly was ready to emerge, were weighed, then dried at  $100^{\circ}\text{C}$ . for 24 hours, and weighed again. The percentage of body weight that was water decreased from an average of 77 per cent in larvae, to about 60 per cent in puparia in which the flies were ready to emerge.

<sup>2</sup>Ages taken from times of leaving back of host.



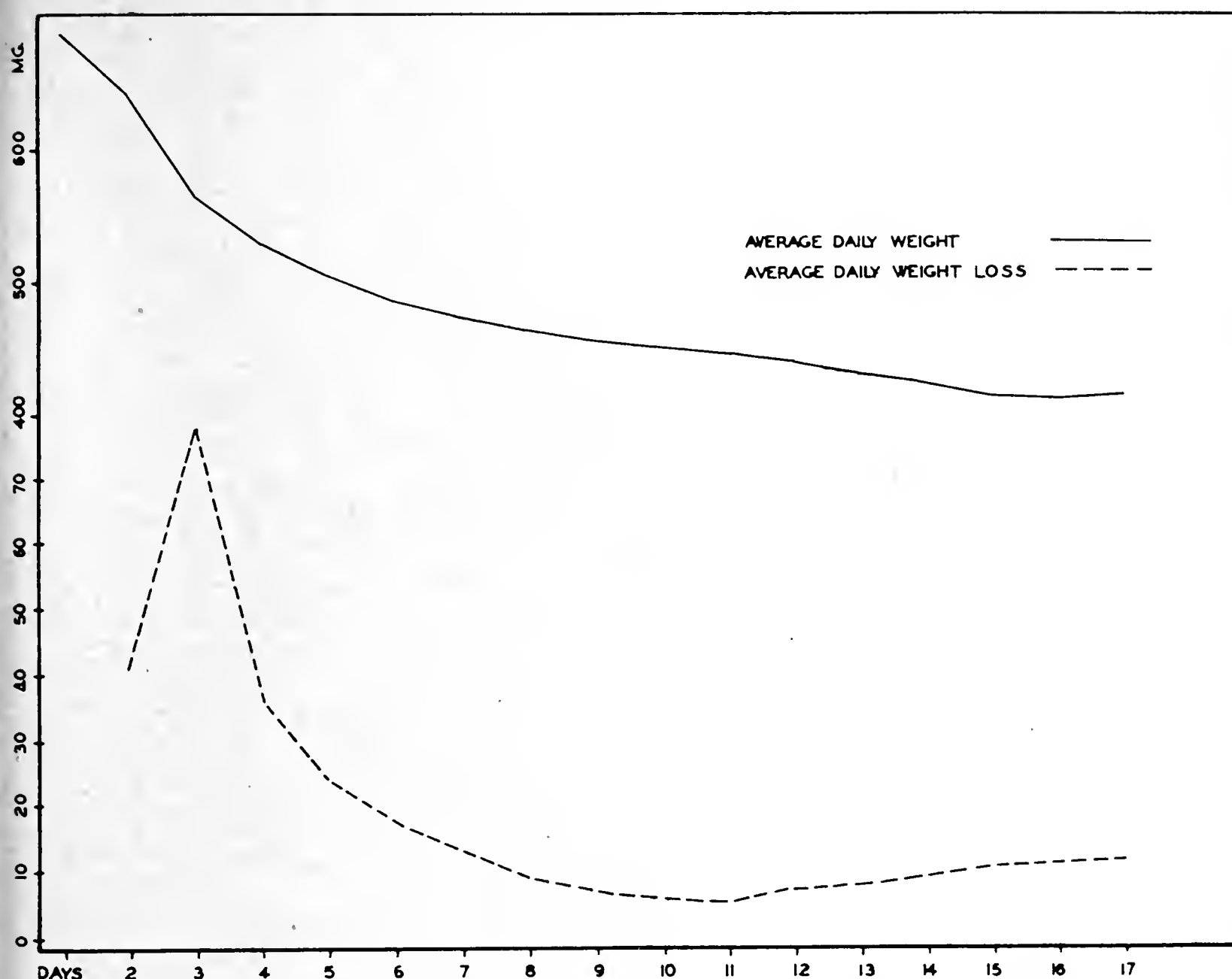


Fig. 4. Average daily weight and daily weight losses of 18 pupae of *Hypoderma lineatum* at 20°C. and 60% R. H.

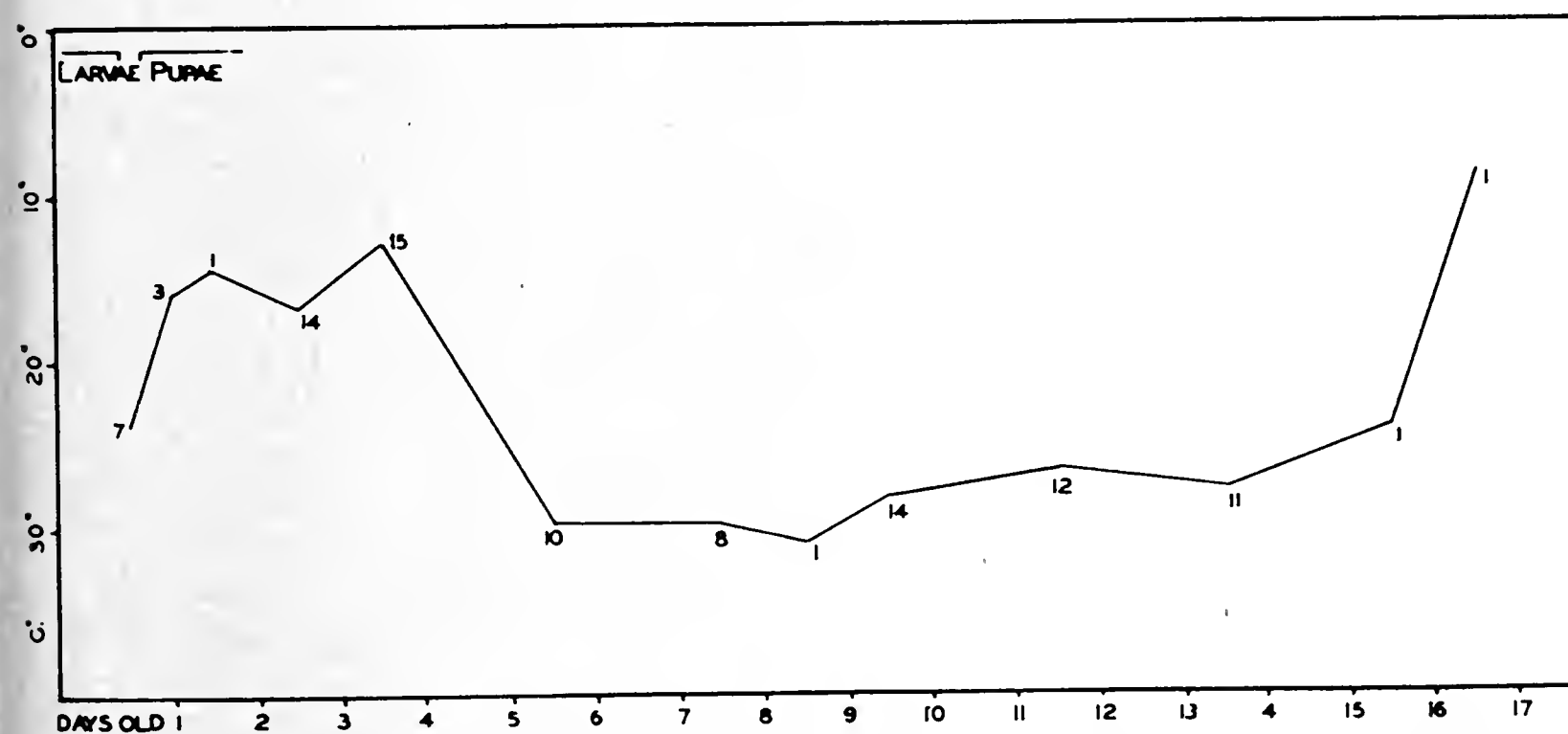


Fig. 5. Average undercooling points of 7 larvae and 91 pupae of *Hypoderma lineatum* according to their ages at 20°C. and 60% R. H.

The undercooling points of 7 larvae and 91 pupae at various ages<sup>3</sup> were determined as described by Salt (1944). All were maintained, as before, at 20° C. and 60 per cent R. H. The data are summarized in Fig. 5. The average undercooling point of -23.7° C. obtained here for larvae agrees closely with that obtained by Salt (1944). But from the time that the puparia are formed, but still soft, until they are 3-4 days old, the undercooling point rises to an average of -15° C; thereafter it falls even lower than Salt's determinations, to an

<sup>3</sup>Ages of larvae were taken from times of leaving host; ages of puparia were known to within 24 hours, as girdles were examined only once daily.

average of  $-28.1^{\circ}\text{C}$ . Apparently during the early development of the pupa, or prepupa, there is a period of about three days when the cold hardiness of the insect is greatly reduced. Fig. 4 showed that this is also the period of maximum weight loss. This comparatively non-cold-hardy period is followed by a much longer period of development during which the insect is at its maximum cold hardiness. The sudden rise to  $-9^{\circ}\text{C}$ . at the end in Fig. 5 was caused by one specimen in which the pupal sac had ruptured in preparation for emergence.

Having established that cold hardiness of the pupa of *lineatum* varied with its physiological age, an experiment was designed to reveal the relationship between survival, age of grub, temperature, and period exposed to temperature. From this it appeared that day-old grubs could not survive a drop to  $-25^{\circ}\text{C}$ . for one hour. In case the sudden change to  $-25^{\circ}\text{C}$ . was responsible for death the test was repeated, lowering the temperature at a rate of  $5^{\circ}\text{C}$ . per hour. Again, none of the larvae survived.

Experiments to discover how and by what route water is lost during pupation showed that it passes through the general puparial wall. Further studies on the changes involved during pupal metamorphosis showed that in *Hypoderma* the sequence is a darkening of the soft third-instar cuticle, contraction of the abdominal tergites, then a general hardening of the integument. In most cyclorrhaphous flies contraction precedes hardening. The observations on daily weight losses and undercooling points suggests the occurrence of a moult within the puparium of *H. lineatum*, during the third and fourth days at  $20^{\circ}\text{C}$ . after leaving the host.

A few attempts were made to discover a means of rearing first-instar larvae *in vitro*. These met with little success; the best medium, raw beef blood serum, maintained larvae alive for only six days.

Observations on the behaviour of laboratory-reared flies yielded results similar to those obtained at Kamloops. Flies placed in a screened enclosure with a calf were lethargic and paid no attention to the animal. It was concluded that the threshold temperature for flight in *H. bovis* is about  $27^{\circ}\text{C}$ . for unmated females, and that protrusion of the ovipositor is a reflex action associated with landing after flight.

### ACKNOWLEDGMENTS

Most of the data embodied in this paper were recorded and compiled by G. P. Holland and J. McLintock; K. Depner assisted the latter. Thanks are extended to all who assisted in observations, collections, measurements, and initial tabulations, and who willingly helped during many early morning hours of fly emergence. These include L. C. Curtis, W. Huxley, Miss G. Hatton, Miss E. Baird, and the late Miss M. Skene of Kamloops, and K. Depner, W. Godlonton, and M. C. Qually of Lethbridge. Much is owed to ranchers of both provinces—notably the Kerr brothers of the Harper Ranch at Kamloops—for their generous cooperation in providing cattle and facilities for the experiments.

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# Some Factors Affecting Populations of *Hypoderma* Species

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## ABSTRACT

The *Hypoderma* population in a large herd of cattle maintained in low animal density on rugged range land was studied in the hypodermal and pupal stages through three successive generations. The behaviour characteristics of individual animals influences sample group selection—randomization is essential. The docility or wildness of animals was not correlated with larval population levels. The standard deviation of larval populations was approximately equal to the mean in sample groups of 7 or 10 animals. A population of 54.6 larvae per animal in the first generation increased to 63.5 in the second generation and declined to 32.0 in the third generation. Mortality was correlated with the incidence of bacterial infection of the cysts and this with larval density. Per cent mortality of *H. bovis* larvae was higher than that of *H. lineatum* and this was correlated with larval density. Predatory birds and rodents devoured up to 95% of pupae and were a constant factor throughout the study. Pupal mortality other than from predation varied highly in different generations and was an important factor in adult density; weather conditions appeared to be an important factor during this stage. As a result of decreased pupal mortality in the third generation, the ratio of adult female survival to the number of animals was higher than in the second generation, although the larval population was reduced to 50%.

## DISCUSSION

W. E. SNOW. How did you determine which portions of the puparial population placed on soil were destroyed by birds, rodents, and trampling?

J. WEINTRAUB. This work was done with puparia planted in the field. Rodents generally chewed the puparia and left the puparial shell behind. Trampled grubs were completely squashed and remained on the spots where they had been placed originally. Previously squashed grubs and puparia were never disturbed. The remainder of the mortality was disappearance of the puparia, which we called bird mortality because puparia were fed to birds in captivity and they left no traces of the grubs.

W. M. ROGOFF. In the predation studies, were the pupae set out in what might be considered "normal" or "natural" settings?

J. D. GREGSON. The situation chosen was a cattle-feeding lot where grubs would normally drop and encounter the predation observed. The grubs were set out in an unmarked random pattern. The site would thus be a natural one.





# The Toxicity of Systemically Active Insecticides Administered to Livestock

By R. D. RADELEFF AND GEORGE T. WOODARD

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## ABSTRACT

Several compounds having systemic insecticidal effects have been found. All save one are limited in application, and the one exception must be investigated further. Dieldrin, aldrin, and lindane were limited by their acute toxicity when administered orally and by the residues left after subcutaneous treatment. Diazinon, Dipterex, and chlordion could be safely used but offered no advantage in parasite control. Pival was of interest as a compound having activity, but its peculiar anticoagulant action made it a hazardous compound and its low insecticidal activity did not recommend it. Dow ET-57 appears to offer real hope in being of relatively low toxicity and good insecticidal action. Residues in tissues must yet be studied.

## INTRODUCTION

The search for systemically active insecticides to be administered to animals has been in progress for the past 30 years (Barrett & Wells 1948, Bishopp *et al.* 1926, Bruce 1939, Knipling 1938, Knipling *et al.* 1948, Lindquist *et al.* 1944, de Meillon 1946, Parman *et al.* 1928), and was stimulated by the successes of de Toledo and Sauer (1950) in Brazil. Since 1950 several compounds have been shown to be effective (Lindquist *et al.* 1953, Lindquist *et al.* 1956, McGregor *et al.* 1954, McGregor *et al.* 1955).

The Animal Disease and Parasite Research Branch and the Entomology Research Branch of the United States Department of Agriculture have cooperated in developing a number of these compounds, the former handling problems of toxicity, methods of administration in screening, treatment of livestock, and tissue sampling for analysis and the latter screening compounds for effectiveness and developing information concerning residues in tissues and products.

At Kerrville, Texas, numerous chlorinated hydrocarbon insecticides were examined by McGregor and Radeleff for systemic activity against the screwworm, *Callitroga hominivorax*. Of the five showing some effectiveness, dieldrin, aldrin, and lindane were selected for further trial against *Hypoderma lineatum* (McGregor *et al.* 1955). At Corvallis, Oregon, Lindquist *et al.* (1953) tested a number of compounds and found dieldrin, aldrin, and lindane more effective systemically against a number of parasites.

At Kerrville, McGregor *et al.* (1954) found that Diazinon (O, O-dimethyl-O-[2-isopropyl-4-methyl-pyrimidyl (6)] thiophosphate), chlordion (O, O-dimethyl-O, 3-chloro-4-nitrophenyl thiophosphate), and Dipterex (O, O-dimethyl 2, 2, 2-trichloro-1-hydroxyethyl phosphonate) were effective against the screwworm in guinea pigs and against grubs in cattle. More recently Dow ET-57 (O, O-dimethyl-O-2, 4, 5-trichlorophenyl phosphorothioate) has shown promise against the cattle grubs, *Hypoderma lineatum* and *H. bovis* (Lindquist 1956).

Chlorinated hydrocarbons, organic phosphorus compounds, and a tertiary valone have been effective. Because the groups create different problems, we shall discuss them separately.

## CHLORINATED HYDROCARBONS

Dieldrin, aldrin, and lindane act upon the central nervous system, eliciting a variety of neuromuscular expressions. The symptoms and resultant lesions have been discussed and summarized in USDA Technical Bulletin 1122 (Radeleff 1955).

Aldrin and lindane were not harmful to cattle at 50 mg./kg. nor was dieldrin at 25 mg./kg., when given subcutaneously as 5-percent solutions in peanut oil. The treatment could be repeated at monthly intervals for 3 treatments with aldrin and 6 with lindane and dieldrin without acute poisoning. Sheep tolerated 50 mg./kg. of lindane and aldrin and 25 mg./kg. of dieldrin, but were poisoned by 100 mg./kg. of lindane. Studies conducted by the Entomology Research Branch and Animal Disease and Parasite Research Branch of the tissue residues in cattle following the subcutaneous treatments showed such quantities present as would prohibit the use of these materials on milk-producing animals and animals

going to slaughter within 2-6 months of treatment. The studies did not, however, preclude the eventual use of these compounds to treat animals such as horses, dogs, cats, and others not normally used for or producing food.

Each of the 3 compounds was lethal to adult cattle when given orally at 25 mg./kg. Cattle tolerated 10 mg./kg. of each of the 3 compounds. Calves were more susceptible to oral dosages, lindane being lethal at 5 and dieldrin at 10 mg./kg. Aldrin was severely toxic to calves at 15 mg./kg. It is thus obvious that these three compounds could not be developed for general use because they produced excessive residues in animal tissues when given subcutaneously and because, when they were administered orally, the insecticidal dose was essentially a lethal one for the cattle.

### ORGANIC PHOSPHORUS COMPOUNDS

The organic phosphorus compounds inhibit cholinesterases of animals. Other biochemical effects probably occur, but they are not well understood. Detailed discussions of the symptoms of poisoning in livestock have been given in USDA Technical Bulletin 1122 (Radeleff 1955). According to available information, most of these phosphorus compounds are rapidly destroyed and eliminated from the body.

*Diazinon* was tolerated by adult cattle at 50 mg./kg. administered subcutaneously as a 5-percent solution in peanut oil. Sheep and goats also tolerated 50 mg./kg. in the same manner. Adult hens were killed by 50 mg./kg. and severely poisoned by 10 mg./kg.

When administered orally, cattle were poisoned by 25 mg./kg. but not by 10 mg./kg. Young calves tolerated 1 mg./kg., but were killed by 10 mg./kg. and were severely poisoned by 2.5 mg./kg.

Because 10 to 25 mg./kg. was required orally or subcutaneously for insect control, *Diazinon* had a reasonable margin of safety, when given subcutaneously, for cattle, sheep, and goats, but not for chickens. The margin of safety did not exist for oral administration to cattle, calves, or chickens.

*Dipterex* was effective when administered orally and appeared to be active systemically when applied to the skin of cattle. Cattle tolerated doses as high as 100 mg./kg., but were poisoned by 200 mg./kg. Sheep tolerated 100 mg./kg. Young calves were poisoned by 10 mg./kg., but not by 5 mg./kg. Calves could be sprayed with 1-percent solutions without apparent harm. Attempts to feed *Dipterex* in mixture with salt produced capricious results.

*Chlorthion* was also effective when administered orally. Cattle and calves tolerated doses of 50 mg./kg. Young calves were poisoned by 100 mg./kg.

These organic phosphorus compounds could be used with safety at the insecticidal dosages, but offered no particular advantage over the more easily practiced spraying, dipping, and dusting.

*Dow ET-57* was announced by the United States Department of Agriculture on May 16, 1956, as being effective systemically against cattle grubs when given orally, killing them before their breathing holes could be cut in the back. Neither the entomological nor the toxicological studies have been published formally, but are being prepared for publication at this time; therefore, it is necessary that we confine our remarks to general statements.

In cattle the initial effect of toxic dosages of ET-57 is to produce muscular weakness and depression. Gastrointestinal disturbances, particularly diarrhea, occur. At the very high dosages (400 mg./kg. and above) there is a second reaction somewhat similar to phosphorus-type insecticide poisoning 6-8 days after treatment.

*Dow ET-57* inhibits cholinesterase of bovine erythrocytes rather slowly over a period of 6-8 days with most dosages. Recovery from the inhibition is very slow.

There is a reasonable margin of safety between the therapeutic and minimum toxic dosages. Oral doses of 100 mg./kg. appear to destroy most of the larvae in the host when administered 2-4 months before grubs normally appear in the back. The minimum toxic dose appears to be 125 mg./kg. Fortunately, there is a very wide range between the minimum toxic and lethal dosages.

In an extensive trial on range cattle, 208 cattle, ranging from 6-month-old calves to aged cows, were given 100-200 mg./kg. of ET-57. None of these cattle were adversely affected by the treatment.

At this time information on residues in animal products is not available; therefore, much experimentation remains before the true toxicological status of this compound is known.

### PIVAL

*Pival* (2-pivalyl-1, 3-indandione) is primarily an anticoagulant and has its greatest application as a rodenticide. It is more toxic in repeated small doses than in single large ones. The compound has insecticidal properties as demonstrated by C. L. Smith at Kerrville in unpublished work.

Sheep tolerated 4 daily doses, each of 10 mg./kg., and were killed by single 100 mg./kg. doses. Chickens tolerated 5 daily doses, each of 25 mg./kg., but were killed by 2 daily doses of 100 mg./kg. Hogs tolerated single 10 mg./kg. doses, but were killed when 10 mg./kg. was consumed over a period of 6 days.

The nature of this paper does not permit us to discuss fully the many toxicological problems associated with the development of systemic insecticides. These have been covered in earlier papers (Radeleff *et al.* 1956a, Radeleff *et al.* 1956b).

The studies discussed in this paper lead us to believe that the use of systemically active insecticides in livestock will soon be practical. As we become more conversant with the problems involved and as links are established in the insecticidal activity mode of action—toxicity—residue chain, progress should become more and more rapid. At this time there are compounds which meet the insecticidal and acute toxicological requirements but which do not meet the requirement of freedom from residues in meat and milk. It should be possible to develop applications of the present compounds in animals not normally used for food and milk production.

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# Report on the Results of Control Methods Applied to Biting Gnats in the Vicinity of Salt Lake City, Utah (Diptera: Ceratopogonidae)

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## ABSTRACT

A program to control the biting gnat *Leptoconops kerteszi* var. *americanus* Carter was initiated in a selected area in the near vicinity of Salt Lake City, Utah, in the spring of 1948. An active control program was continued until the fall of 1952. Since then the area has been under observation. The life history and habits of the gnat were studied and various methods for the control of all stages were used including water management, soil cultivation, land fill and chemical applications by ground and air equipment. Repellents and special clothing were also used to prevent the attacks of these gnats.

A chemical soil sampling method to determine the presence of gnat larvae was developed which makes it possible to rapidly conduct sampling in the field. It was determined that water management, plowing, filling, and the application of chemicals can be effectively used as source reduction methods. The most effective control was obtained by reducing the water content of the soil. DDT spray applied at the rate of one pound of DDT per acre as a direct larvicide, or at two pounds per acre as a pre-hatch larvicide produced effective results. The residual effects of DDT at concentrations from 1 to 10 pounds per acre were apparently not retained after the second year. Chemical adulticides applied as mists, sprays or smoke aerosols proved effective from four hours to several days. Hoods for protecting the head were effective but uncomfortable. Repellents used were unsatisfactory but some of the more recently developed repellents show promise. Adult gnats began to reappear in the area in 1955. A few larvae were found in the soil in the spring of 1956 and adults were present in sufficient numbers to be annoying.

## INTRODUCTION

The biting gnat *Leptoconops kerteszi* var. *americanus* Carter is an extremely abundant and annoying pest in many parts of Utah and adjacent states each spring during a period of about six weeks. In areas where these gnats are abundant it has been estimated that they reduce the working efficiency of men exposed to their attack by 10 to 20 per cent. The bite of these insects is almost painless when inflicted but an inflamed swelling soon appears, accompanied by irritation and itching which may continue to annoy the victim for 72 hours or longer. In extreme cases medical attention and hospitalization of the victims have been required.

The first known attempt to conduct a control program to protect workmen from the attacks of these gnats was started in Salt Lake City, Utah, in 1948, on the property of the Salt Lake Refining Company, a subsidiary of the Standard Oil Company of California. The gnat control program was planned and conducted by D. M. Rees and J. V. Smith, of the University of Utah, for the Salt Lake Refining Company. The control measures were continued successfully until 1952 when they were considered no longer necessary on this property. Rees and Smith (1950, 1952) reported on this work.

Following the success of this program attempts to control these gnats have been made at the Tooele Ordinance Depot at Tooele, Utah, and on construction projects in other parts of Utah by the Stauffer and the Morrison-Knudsen Construction Companies and others.

## LIFE HISTORY AND HABITS

Eggs recovered from dissected females varied in number from 35 to 65 with an average of approximately 50 per female. These eggs are apparently deposited on suitably moist soil. The larvae have not been detected in the soil until the following spring when they have been recovered about six inches beneath the surface, in soil which is saturated with moisture. The larvae usually appear in early March, and a slow migration of the larvae takes place towards the surface where they pupate on the surface of the soil in early April. In about 10 days after pupation the adults emerge. The adult gnats are on the wing for

approximately six weeks during April, May, and June, the time of emergence varying as much as three weeks depending upon variations in climatic conditions. As many as 5,710 adult gnats emerging from the ground were collected in one season in a trap covering three square feet of surface area. This is the equivalent of an emergence potential of approximately 83,000,000 gnats per acre.

The female gnat feeds only during the daylight hours on warm calm days. If undisturbed it requires about two to seven minutes to complete a blood feeding. On man they feed on any exposed area of skin but prefer to bite where wearing apparel touches the body and at the margin of the hair line on the head.

Twenty-four hours after emergence mating swarms consisting principally of males and numbering several hundred individuals have been observed near the breeding areas. Other swarms, consisting mainly of females, form readily near available hosts when climatic conditions are favorable. The flight range is unknown but gnats have been annoying at least a mile from any possible developmental habitat.

### INSPECTION

Before source reduction methods or larvicides can be effectively applied the area in which the gnats develop must be determined. This can be done only by locating the larvae in the soil and was first accomplished by obtaining soil samples which were washed and screened to ascertain if larvae were present. The samples had to be taken during the limited period when the minute larvae were in the upper three inches of the soil, usually in late March. This was a slow laborious method and greatly reduced the area that could be sampled for larvae. A method of larval sampling was later developed during this investigation which made it possible to sample large areas rapidly and accurately. It was determined that when the larvae are near the surface of the soil just prior to pupation they will become extremely active if they are treated with a solution of DDT or other organic insecticides commonly used as larvicides. By treating the surface of foot-square sample soil plots at the proper time with a  $2\frac{1}{2}$  per cent solution of DDT and then carefully examining the soil about five to eight minutes after treatment, the tiny reddish larvae, if present, can be seen writhing on the surface of the soil. The boundaries of gnat-producing areas can readily be established by this method and treatment applied only to gnat-producing soil.

### CONTROL

#### SOURCE REDUCTION

Since 1948 various types of control methods have been applied successfully in the field in Utah to control these gnats. Like similar insect control problems, source reduction has proved to be the most effective method but it is not always the most practical.

It was determined that a reduction in the early spring of the soil moisture to 25 per cent saturation or less will prevent the gnats from emerging as adults. It also was demonstrated that, in certain situations, covering the area where gnats develop with two feet or more of top soil will eliminate gnat production. The turning under of the top soil by deep plowing in the fall of the year when the soil was dry has eliminated gnat production in the plowed area for two years or more. Intensive harrowing and disking of the soil when dry greatly reduced gnat production the following season but this method proved effective only when repeated every two years.

#### LARVICIDING

The larvae of *Leptoconops kerteszi* var. *americanus* were found to be susceptible to all of the common organic insecticides tested. It was determined that 1 lb. of emulsified DDT applied in 28 gal. of water per acre was effective in destroying the larvae and pupae in the soil. This larvicide was found to be most effective if applied when the larvae are concentrated near the surface of the soil just prior to pupation. The larvicides can be applied effectively by air, ground, or hand equipment, although at the rate of 28 gal. per acre application by air is impractical. Application by heavy ground equipment on saturated soil was not always possible and application by hand with knapsack spray pumps was slow, laborious, and expensive. An attempt was made, therefore, to apply the larvicide in the fall of the year with ground equipment when the soil was dry. DDT applied at the rate of 2 lb. per acre in 28 gal. of water proved to be effective for two years in preventing gnats

from developing in an area. Higher concentrations of DDT up to 10 lb. per acre applied on experimental plots did not remain effective longer than two years. At present, plans are in progress to use dieldrin on gnat producing soil at the rate of 1 lb. per acre, in an attempt to extend the effectiveness of pre-hatch soil treatment for more than two years.

#### ADULTICIDING

Residuals applied for adult gnats with the same chemicals and concentrations used for mosquitoes are effective only when the gnats come in contact with the chemicals. As the gnats rarely enter or rest on buildings or other man-made shelters, residual adulticides are of little value as a control measure. Adults can be readily destroyed by sprays, mists, and aerosols of DDT, heptachlor, dieldrin and similar insecticides. The same amounts of these insecticides used as adulticides on mosquitoes are effective on gnats. They have been applied effectively by airplane and ground equipment such as knapsack and power spray pumps, Buffalo Turbine, TIFA, and smoke aerosol generators. Adulticiding for gnats seems to have a slightly longer effectiveness than when adulticides are applied on mosquitoes but applications must be repeated periodically as the gnats reappear. Adulticides properly applied as mists, sprays, or smoke aerosols are effective from four hours to several days depending on the source of the gnats, climatic conditions, and the period of the gnat producing season when the adulticide is applied.

Gloves and a head net with 32 or more mesh openings per square inch will provide protection from the gnat bites but are uncomfortable and cumbersome. Numerous repellents have been tested and most of them have proved to be unsatisfactory. Some of the more recent repellents such as "Pellent", distributed by the Andy Lotshaw Company, showed promising results during the spring of 1956.

#### DURATION OF CHEMICAL CONTROL MEASURES

The last application of insecticide on the property of the Salt Lake Refining Company was made in November, 1952. This was a pre-hatch treatment applied by ground equipment on 44 acres of persistent and prolific gnat producing soil. The treatment consisted of 2 lb. of emulsified DDT in 28 gal. of water per acre. In the spring of 1953 and again in the spring of 1954 adult gnats were not detected in the area or in the immediate vicinity. Larval sampling was not conducted on the area during these two seasons.

In the spring of 1955 some random larval sampling was conducted with negative results but a few adult gnats were present in the area during May. In the spring of 1956 a few gnat larvae were detected in the soil by larval sampling and a few adult gnats were collected in three of the six adult gnat traps operated on the breeding ground during the gnat season. Adult gnats were also present in the area and were reported as particularly annoying from May 14 to 21, 1956. Apparently this pre-hatch treatment of 2 lb. of DDT per acre applied in the fall of 1952 remained effective for the following two springs. In 1955, the third year, a few adults were present and in 1956, the fourth season after treatment, larvae were recovered from the soil and adults were present in sufficient numbers to create an annoyance.

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 Rees, D. M., and J. V. Smith. 1950. Effective control methods used on biting gnats in Utah during 1949. (Diptera: Ceratopogonidae). *Mosquito News* 10(1): 9-15.  
 Rees, D. M., and J. V. Smith. 1952. Control of biting gnats in North Salt Lake City, Utah (Diptera: Heleidae). *Mosquito News* 12(2): 49-52.

#### DISCUSSION

A. W. A. BROWN. Would it be of interest to test the resurgent population of *Leptoconops* for their resistance to DDT? It has often occurred that a population reduced to a very low level by chemical control comes back resistant to that insecticide.

D. M. REES. I think the suggestion is a good one and will consider making such a test. We plan to proceed to dieldrin as a means of control.

E. KIRCHBERG. Can you give information about the flight range of the biting gnats? This information appears to be important in relation to the limits of control measures.

D. M. REES. The biting gnats have been found at least a mile from their nearest developmental area. Their flight range is undoubtedly greater than a mile.

P. A. Woke. How long do adults remain in the breeding area following emergence?

D. M. Rees. Some may remain as long as 30 days which is their approximate life span. Others seem to move into the area in about one or two days if the local population is destroyed with an adulticide.



# Production and Control of Floodwater Mosquitoes Incidental to Water Level Operations on Reservoirs of the Tennessee Valley Authority

By W. E. SNOW

Division of Health and Safety, Tennessee Valley Authority  
Wilson Dam, Ala.

## ABSTRACT

As the marginal growth band has become more stabilized along the main river reservoirs due to regularity of water level operations, the floodwater mosquito *Aedes vexans* has become well established in the zone just above top summer pool level in certain reservoir areas. This zone is surcharged in the spring to strand flottage as a malaria control operation and incidentally hatches an initial brood of floodwater mosquitoes. If the surcharge phase and recession to top pool level occur over a period of about 5 days, many larvae in grassy, well-drained situations become stranded in the vegetation. On the other hand, if water remains in the surcharge zone for several weeks, emergence of *A. vexans* generally occurs. The weekly cyclical fluctuation and seasonal recession phases of the malaria control program are especially favorable for the control of floodwater mosquitoes. Even when rains coincided with the upper end of the cycle and hatching in marginal grass occurred, scheduled recession over the next few days was sufficient to eliminate larvae by stranding. On many of the reservoirs in the tributary watershed, seasonal water level recession is a regular operation whereby stored water is gradually released during the drier periods in summer and fall for downstream use. A recession rate of 0.2 to 0.3 ft. per week beginning shortly before July 1 has been found adequate for mosquito control. When stored water is allowed to remain in the marginal growth band for several weeks in the spring before recession is begun, small numbers of *A. vexans* usually emerge. In instances when the growth band has become periodically flooded during the summer with intermediate drawdown periods of several weeks intervening, very large populations of *A. vexans* have been produced.

## GENERAL

In connection with development of water level schedules on the Tennessee River system for the primary purposes of flood control, navigation, and production of power, consideration was also given from the very beginning to water level management for control of malaria mosquitoes. The desirable phases of water level management for malaria control now being used on the main river reservoirs include (1) a rapid flood surcharge phase coincident with spring filling for stranding of drift and flottage, (2) a constant pool phase in late spring and early summer to discourage or delay the development of marginal vegetation, (3) the cyclical fluctuation phase initiated when moderate anopheline production begins and used primarily to interrupt marginal breeding during the drawdown phase of the cycle, and (4) the seasonal recession phase which is used on main river reservoirs in combination with cyclical fluctuation during the latter part of the growing season at a rate of about 0.2 foot per week. On Kentucky Reservoir and the storage reservoirs where weekly cyclical fluctuation is not practical, recession is begun earlier in the season with a faster rate of drawdown.

While the water level management program mentioned above was developed mainly for the control of *Anopheles quadrimaculatus*, a permanent pool type of mosquito, it has incidentally controlled production of some floodwater mosquitoes by stranding under certain conditions. In a few instances, on the other hand, it has greatly enhanced production of floodwater species. It should be pointed out that current impoundage laws of the Valley states require control of only the malaria mosquito, *A. quadrimaculatus*. In March 1956, however, TVA adopted a pest mosquito code whereby control of floodwater mosquitoes is undertaken in portions of certain reservoirs that are the principal source of pest mosquito annoyance in the area. It is the purpose of this presentation to show favorable and unfavorable aspects of water level schedules for propagation of floodwater mosquitoes.

The floodwater species of mosquitoes (*Aedes* and *Psorophora*) deposit their eggs on the soil in grassy depressions, low shaded situations, and along shorelines that are intermittently flooded. After a variable period of embryonic maturation and conditioning, these eggs will hatch if flooded under favorable conditions; otherwise, they may remain viable

on the soil for at least one year. When suitable conditions prevail, nearly all hatching occurs in the first 24 hours of wetting. Adults of *A. vexans* have appeared as early as 18 days after flooding of the breeding area during the last week in March. In mid-summer adult *Psorophora confinnis* and *P. cyanescens* have emerged in 5 days after breeding sites were filled with rainwater. Time of year, therefore, is an important factor in determining how long water may be stored in certain reservoir situations without becoming a mosquito hazard.

### MAIN RIVER RESERVOIRS

As the marginal growth band has become more stabilized along the main river reservoirs due to regularity of water level operations, floodwater mosquitoes have become well established in the zone just above top summer pool level or particularly over the contours covered by water in the surcharge phase. Thus, each spring when filling and surcharging ordinarily take place in April (Kentucky Reservoir is scheduled to surcharge the first week in May), a general hatching of *A. vexans* occurs along suitable portions of the reservoir margin. The fate of this initial generation is largely dependent upon the surface conditions of the surcharge zone and the duration of the surcharge phase.

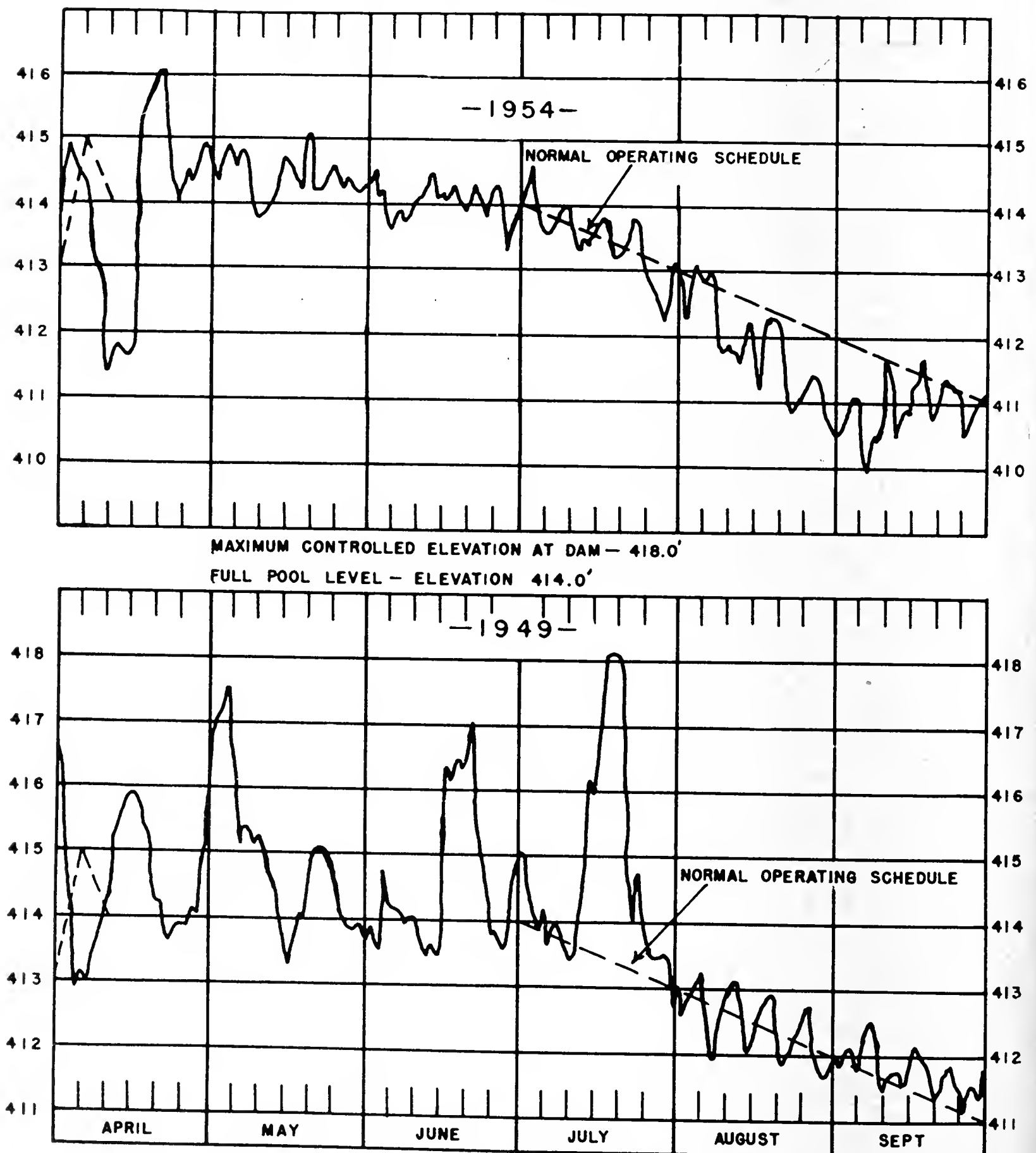


Fig. 1. Water level operations Pickwick Reservoir, Florence, Alabama, during the growing season of 1949 and 1954.

A desirable rule curve for suppression of floodwater mosquito development on the main river reservoirs occurred over the 1954 growing season on Pickwick Reservoir (Fig. 1). In the McFarlands Bottom area of this reservoir surcharging during the April 16–19 period followed by a relatively rapid recession from April 20–22 (0.5 to 1.0 ft. in 24 hours) was responsible for stranding out the majority of aedine larvae in the grassy surcharge zones at contours 415.0 to 416.05 ft. along the outer margins of willow-lined drainage ditches. Larval densities were estimated on the morning of April 21 by dipping in four marginal grassy stations between elevation 415.0' and 416.0' and in two stations beneath willow in the drainage ditch at elevation 414.5'. Based on 10 dips per station with a standard white enamel dipper, grassy stations averaged 1.2 second stage, 55.8 third stage, and 10.9 fourth stage larvae of *A. vexans* per dip. In the main ditch below willow, larvae averaged 0.2 second stage, 2.5 third stage, and 1.2 fourth stage per dip. Larvae of *A. vexans*, *A. trivittatus*, *P. ferox*, and *P. ciliata* were mainly present beneath the willow. The water level receded rapidly in the ensuing 24 hours, dewatering the 415.0 to 416.0 ft. contour and stranding most of the larval mosquito population. Water levels fluctuated generally between 414.0 to 415.0 ft. until the first week in July when cyclical fluctuation and seasonal recession of 0.2' per week began from elevation 414.0'.

It might be expected that if all eggs wetted in the surcharge zone hatched and the larvae became stranded no further hatches would result from later floodings. Several conditions tend to upset this assumption. In the first place floodwater mosquitoes frequently emerge at lower contours or wander in from outside areas and "reseed" the surcharge zone. Also, eggs of certain floodwater species, particularly *P. cyanescens*, do not appear to be "conditioned" to hatch early in the season (April) when surcharging occurs and eggs of *A. vexans*, *A. sticticus*, and *A. trivittatus* hatch. Artificial and natural flooding of experimental basins on Pickwick Reservoir in late March and early April 1956 produced early generations of *A. vexans*, *P. discolor*, and *P. ciliata*; however, no *P. cyanescens* were detected then. Intermittent rains from April 5 to July 19 were insufficient to produce further series of mosquitoes from these basins. Heavy rain on July 19 hatched sizable numbers of *P. cyanescens* from these basins, but no other species were detected. As many as 50 feeding females could be counted on the forearm after a 30-foot walk through grass at the edge of the basin on the afternoon of July 25.

Referring to water level operations of Pickwick Reservoir for the summer of 1949 it will be seen that the surcharge zone was reflooded three times in the season after the initial spring filling and regular surcharge phase in mid-April. The return to full pool level was particularly slow during the first half of May, giving the mosquitoes surviving the dewatering of May 4–6 ample time to emerge. Flooding of the surcharge zone over the period July 12–23, 1949, was particularly favorable for heavy hatching of floodwater mosquitoes because it not only came at a suitable warm period in the season for hatching and rapid development of all species, but flooded contour 417.5 to 418.1 ft. which had not previously been flooded in 1949. Considering five days as a minimum for completion of the immature stages, multitudes of mosquitoes were able to emerge. Annoyance to townspeople by *P. cyanescens* in Florence, Sheffield, and Tusculumbia, Alabama, was so intense that these cities contracted with a private firm to space treat the towns with DDT by plane. It may be seen, therefore, that sharp deviations from the rule curve during the constant pool phase or later can become very favorable for floodwater mosquito production, especially if water remains in the surcharge zone as long as a week.

During the summer, Ft. Loudoun Reservoir is scheduled to fluctuate between elevation 812.0' and a top summer pool level of 813.0' once each week as a part of the malaria control program. Rains on August 28, 1954, coupled with water elevations during the peak of the cycle, hatched eggs of *A. vexans* in a willow swale near Rockford, Tennessee, on the Little River embayment over the weekend of August 28–29. Small stage larvae, 30–50 per dip, were found between contour elevations 812.6 to 812.9 ft. on August 30. During the draw-down phase of cyclical fluctuation from August 31–September 3 the aedine larvae were stranded in marginal grass. A field check on September 3 disclosed only one mature larva in an isolated micropool on the clean shoreline exposed at the lower end of the cycle. Cyclical fluctuation of one foot per week on well-draining shorelines appears to be ideal for suppression of floodwater mosquito propagation, for, even though hatching may occur when the upper end of the cycle is exceeded, dewatering is so rapid that larvae are soon stranded or drawn into open water where life is hazardous.

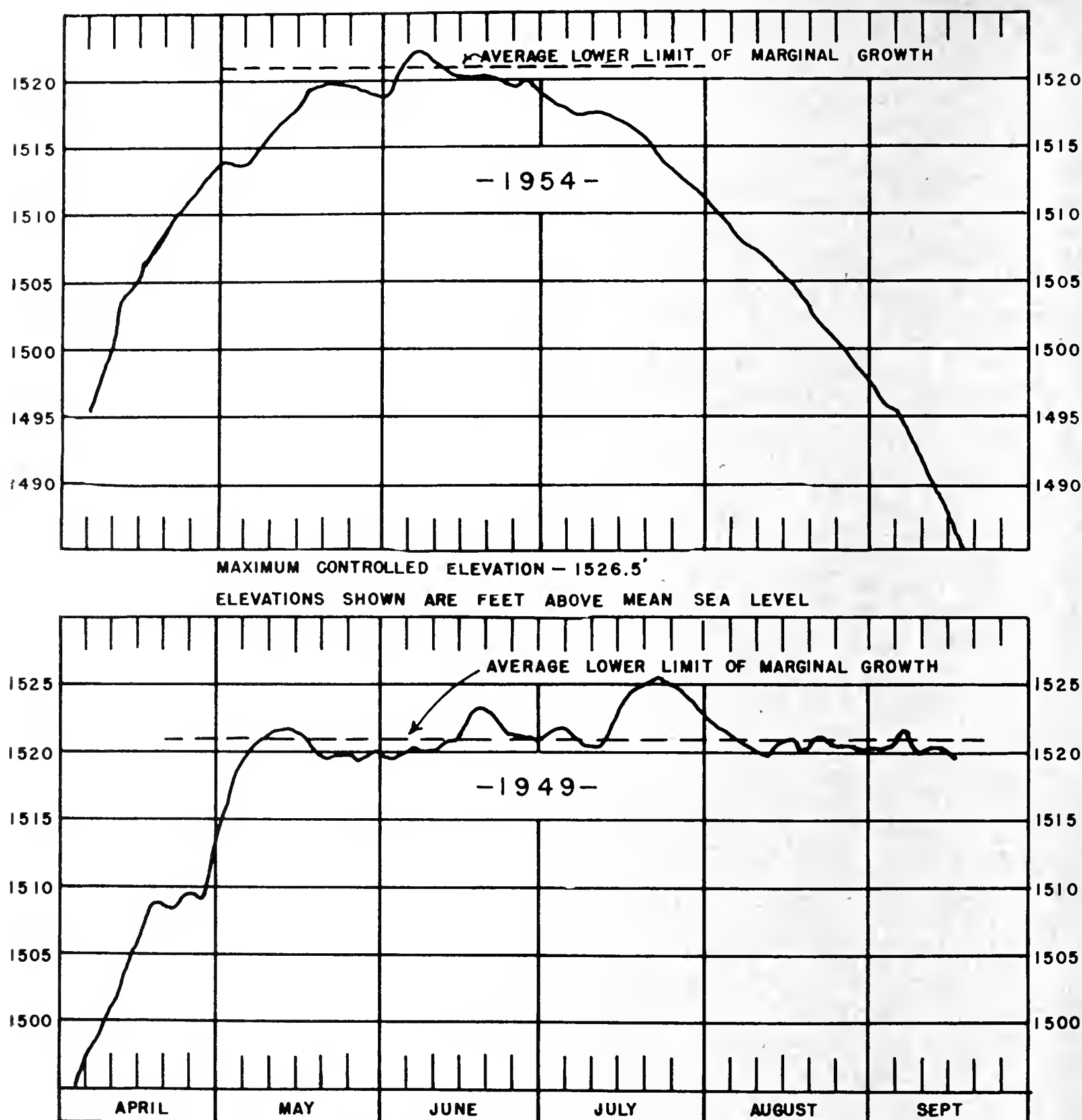


Fig. 2. Water level operations Hiwassee Reservoir, Murphy, North Carolina, during the growing season of 1949 and 1954.

### STORAGE RESERVOIRS

On many of the reservoirs in the tributary watershed, seasonal water level recession is a regular operation whereby stored water is gradually released during the drier periods in summer and fall for such downstream uses as power, flood control, navigation, irrigation, and domestic or industrial water supply. Seasonal recession may be effective for mosquito control if the time of beginning and the rate of fall can be adjusted to meet the need. The storage reservoirs are usually filled during the seasonally wet period from late winter through spring and drawdown is generally started at the beginning of the dry season in early summer. A recession rate of 0.2 to 0.3 ft. per week beginning before July 1 is considered adequate for mosquito control. The water level operation curve occurring on Hiwassee Reservoir at Murphy, North Carolina, in 1954 shows a desired schedule of operation suitable for control of both anopheline and culicine mosquitoes occurring in the area although a slightly sharper peak would be better for control of *A. vexans* (Fig. 2). Water was in the lower portion of the marginal growth band (contour 1521.0 to 1522.4 ft.) for about one week (June 4-11) and hatched eggs of *A. vexans* present in this zone. Recession was rapid enough at this time of year to strand most of the larvae and pupae on the margin or carry them into the main channel. As a rule water temperatures on Hiwassee Reservoir at this period of the year are in the low 70's and mosquitoes would not generally appear in



less than 10 days. However, because some suitable breeding areas along the reservoir margin exist at contours below elevation 1521.0', earlier hatching of *A. vexans* occurs and adults are able to emerge before recession can be effective. A plot of soft rush (*Juncus effusus*) at contour 1517.0 to 1518.0 ft. along the Valley River arm of Hiwassee Reservoir at Murphy, North Carolina, has been found annually to produce an early spring brood of *A. vexans* before reservoir water reaches the main band of marginal vegetation. Consequently, these mosquitoes emerge and tend to reinfest the general area though at a low level of egg deposition. This is apparent when annual broods of *A. vexans* hatch at contours 1521.0 to 1522.0 ft. and higher and the previous year's hatch from these contours is known to have been destroyed by stranding and larviciding. It is assumed that all viable eggs of *A. vexans* will hatch when wetted under favorable conditions.

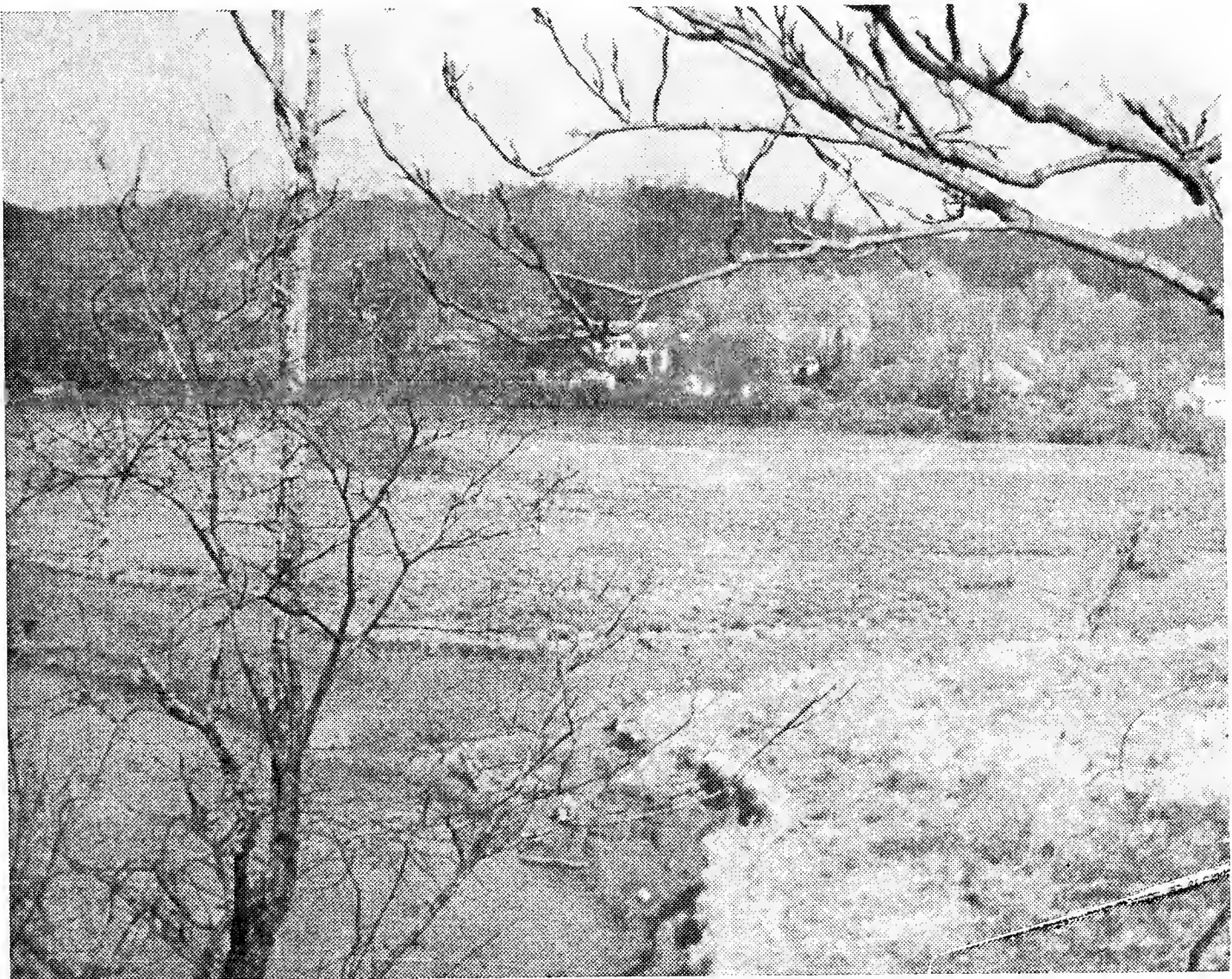


Fig. 3. Breeding area of *Aedes vexans* in the McCall Bottom, Hiwassee Reservoir, Murphy, North Carolina, April 13, 1953.

Occasionally it has been necessary to hold water in storage reservoirs at elevations well within the marginal growth band for periods which permit the emergence of floodwater mosquitoes. If several weeks then intervene during which time new egg deposition occurs, a subsequent flooding may produce sizable numbers of mosquitoes. An examination of the water level operations for Hiwassee Reservoir in 1949 indicated a very favorable schedule for floodwater mosquito production (Fig. 2). Schedules for the preceding two years had been similar, consequently a sizable population of *A. vexans* was ready to hatch when conditions became suitable. A heavy hatch occurred along channel flats of the Valley and Hiwassee Rivers at Murphy, North Carolina, between contours 1520.0 to 1521.5 ft. during the early part of May 1949 (Fig. 3). Due to incomplete drainage an initial heavy brood emerged during the latter part of May. The inside of a white enamel dipper could be covered with adult *A. vexans* with one sweep through marginal soft rush. Townspeople at Murphy were greatly annoyed and no nighttime recreation was possible for two weeks. Subsequent series of *A. vexans* appeared as higher elevations were reached in the rises of June 15–20 and July 13–23. Only through concerted control efforts of city officials and TVA workers were excessive populations prevented from emerging after these summer floodings. Currently, a program of surveillance and treatment with DDT larvicide is being used at Murphy. After water levels have reached the 1521.0 to 1522.0 ft. contour interval in the spring, field inspections are made for floodwater mosquito larvae and DDT applied

as necessary. The possible use of dieldrin as a preflood treatment for floodwater mosquitoes is now being investigated on both main river and storage reservoir margins. Results obtained in the 1956 season show it is very effective against *A. vexans* at the rate of 1 lb. per acre on vegetated margin.

Due to the variability of late winter and early spring rainfall, certain storage reservoirs (Fontana, Watauga) with wide zones of fluctuation do not always reach top pool elevation in the annual spring filling operations. Thus, floodwater mosquitoes have slight chance to become established in any particular marginal zone since reservoir water may reach the upper limits only once in several years. As a rule these reservoirs are drawn rather heavily in the summer and fall and no further opportunity for mosquito breeding occurs until spring filling takes place the next year.

# Algunas Observaciones Sobre el Control del Tórsalo<sup>1</sup> en Costa Rica

Por EVARISTO MORALES M.

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## ABSTRACT

The tórsalo, *Dermatobia hominis* (L.), is one of the most important pests affecting cattle in Costa Rica. Little is known about its origin in the country. Its distribution has been noted from 0 to 1200 meters above sea level, and it prefers wooded and damp areas with higher gradation in the northern part of the Alajuela and Limón provinces. From 30 to 35% of all skins produced are damaged. One-third of all cattle are either infested or in infested zones. Control tests have been conducted using chlordane in concentrations of 0.5%, 1%, 3% and 4% (suspension). Best time for application—two weeks during rainy season and three weeks during dry season. The smaller concentration is the best. No signs of intoxication. Toxaphene, emulsifiable and wettable powders, and lindane wettable powder were tested as sprays as well as by immersion using wettable powders of both. Lindane was used as a smear at 3%. All gave promising results. To obtain effective control, pasture and grazing land must be kept free of weeds, especially tall weeds. Insecticides of long residual effect must be used in stables to control the adults. A campaign conducted throughout the country, using all available resources, could bring this pest under control.

Cuando nos detenemos a pensar en cual es una de las más importantes plagas en la ganadería de Costa Rica, concluimos en que es el "tórsalo" y, nos atrevemos a afirmar que en muchas de nuestras zonas ganaderas esta plaga es determinante del buen éxito o del fracaso de esta rama de la agricultura y que sin este azote, más leche, más carne y mejores pieles habría para más gente.

Quizá lo mismo sucede en el resto de América Tropical, desde Mexico hasta el Norte de la Argentina y algunas islas del Caribe.

El insecto a que nos referimos ha despertado el interés de científicos y técnico desde 1781, año en que Linnaeus lo describió como *Oestrus hominis*, (N. Nord. Beytr. Phys. Geogr. Erdk. I. 157. 1781.) y siguiendo ese interés, en años posteriores han ido apareciendo diversos estudios, dando motivo a un número variado de nombres científicos, hasta llegar al que en la actualidad ostenta.

El hecho de que la hembra se vale de vectores para el transporte de sus huevos y de esa manera perpetuar la especie, no fue conocido sino hasta 1910, descubierto por los Dres. Morales y Núñez Tovar, de Guatemala y Venezuela, respectivamente.

Al servirse ella de una variedad de vectores (mosca de establo, mosca doméstica, zancudos (*Psorophora* (*Janthinosoma*) *lutzi*, *P. ferox*, *Culex spp.* y garrapatas) para proseguir su procreación abre la posibilidad de tener entre los hospederos definitivos a una gran variedad también de especies de vertebrados (el hombre, con preferencia los niños, relacionado con la población de mosca doméstica, bovinos, venados, ardillas, raramente caballos, algunas aves, muy pocas veces los cerdos, etc.).

El Dr. D. Carbonell (1938) en su "Parasitología en Venezuela y los trabajos del Dr. M. Núñez Tovar", transcribiendo lo escrito por Tovar, dice:

"Nuestras pacientes investigaciones entomológicas llevadas a cabo en el Estado de Monagas durante los años de 1910 y 1911 nos permitieron establecer de una manera rigurosa la afinidad parasitaria que liga los braquíceros con los nemóceros, en lo que respecta al complemento de maternidad que prestan los mosquitos (*Janthinosoma lutzi*) a las larvas de un múscido (*Dermatobia cyaniventris*).

"Dejó igualmente establecido este nuevo papel patógeno de los mosquitos, los cuales debemos considerar no sólo agentes transmisores de las enfermedades citadas, sino tambien como vehículos animados que transportan y depositan sobre la piel (hombre o animal) del segundo huésped intermediario, las larvas cutícolas de la mosca *D. cyaniventris*. Nos permite asegurar de un modo positivo que dicha mosca no pone sus huevos, según lo asientan Blanchard, Le Dantec, Brault, Jaenselme y Rist, Brumpt, etc, sino que los deposita sobre el cuerpo de un mosquito del Género *Culex*, donde permanecen adheridos hasta que se transforman en pequeñísimas larvas que a su vez se adhieren a los músculos torácicos del zancudo, para luego introducirse, vehiculados por éste, bajo la piel del

<sup>1</sup>*Dermatobia hominis* (Linn.) Familia Cuterebridae, Orden Diptera. Comunmente llamado en Costa Rica Tórsalo o Gusano de Monte. En USA: Human botfly. En otros países: Berne, moyocuil, colmoyote, ura, nuhe, etc.

hombre o animales vertebrados, de donde caen espontáneamente sobre la superficie de la tierra tan pronto como llegan a su estado de madurez o completo desarrollo.

"El hecho fue notificado a nuestros colegas Razetti y luego a Blanchard.

"Es oportuno advertir que todos los ejemplares de mosquitos portadores de huevos que hasta hoy hemos logrado examinar, además de ser hembras, pertenecen siempre a un mismo Género y a una misma especie".

### DISTRIBUCION

La distribución geográfica en el país es bastante extensa, como puede ser notado en el gráfico (Fig. 1), que se incluye en este trabajo. Vive desde 0 metros hasta 1200 a 1.300 m.s.n.m.

Más arriba de esas altitudes su presencia es muy rara y, aun dentro de esos límites su gradación varía grandemente en las zonas de infestación, ya que prefiere aquellos lugares boscosos, donde existe la humedad apropiada y, muy raramente puede ser visto en o cerca de los establos o de las habitaciones humanas.

Algunas zonas, como las del Pacífico, son afectadas en un porcentaje bastante bajo; pero aquellas de la Meseta Central y Norte y Oeste del país, lo son en alto grado.

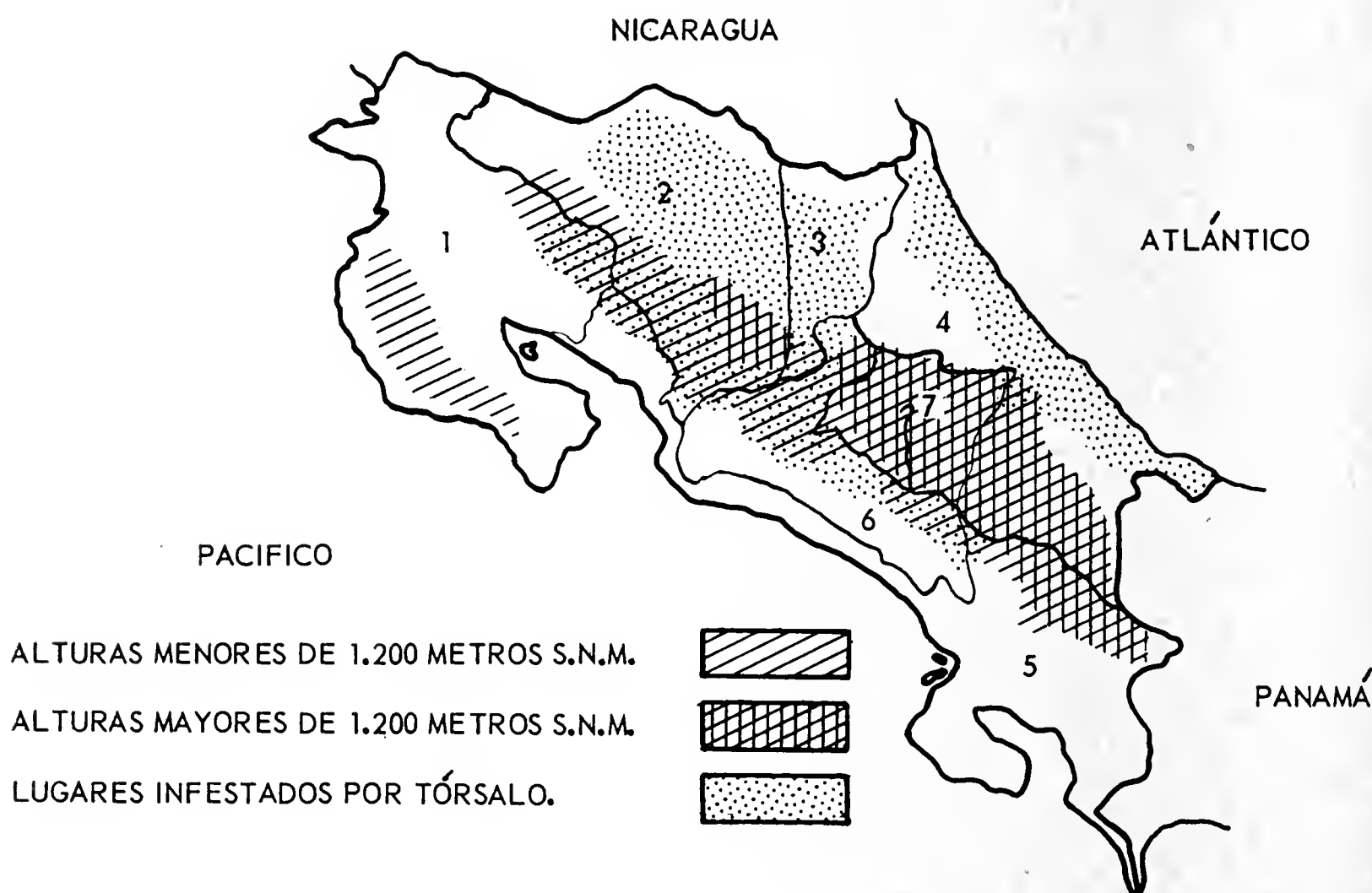


Fig. 1. La distribución aproximada de tórsalo en Costa Rica. Provincias: 1, Guanacaste; 2, Alajuela; 3, Heredia; 4, Limón; 5, Puntarenas; 6, San José; 7, Cartago.

### ORIGEN

El origen del tórsalo en Costa Rica es hasta cierto punto incierto en el presente y aunque el Dr. Pittier lo cree nativo en Costa Rica, otros autores lo juzgan procedente de Venezuela, traído junto con ganado infestado.

### IMPORTANCIA ECONÓMICA

La importancia económica de la plaga es enorme y sus daños estan, es claro, en relación a la población ganadera existente en los lugares infestados. Un dato que puede indicar la importancia de ella es el de que sólomente los cueros infestados, o en otras palabras, la pérdida en los cueros puede alcanzar a un 30%. Debemos pensar en las pérdidas en leche, carne, infestaciones por otros parásitos, etc., y tendremos un cuadro desastroso.

Este país con su ganadería sumamente valiosa, dado lo floreciente de la misma, debe entrar en un plan extensivo de control, para lograr erradicar la plaga, ó, por lo menos controlarla.



Esta ganadería aunque pequeña es de mucha importancia en la economía del país y, se encuentra distribuída más o menos en la siguiente manera:

*Guanacaste . . . . .	248.900	reses
**Alajuela . . . . .	146.150	"
*Puntarenas . . . . .	105.000	"
**San José . . . . .	72.600	"
Cartago . . . . .	48.150	"
**Limón . . . . .	19.500	"
**Heredia . . . . .	16.300	"

\* muy poca infestación.      \*\*Alta infestación.

El cuadro anterior indica que por lo menos 1/3 del total de nuestra ganadería se encuentra infestada o en zonas infestadas.

## CONTROL

Hasta hace pocos años el control de la plaga había sido difícil de lograr y, aunque el hombre desde que tuvo conocimiento del problema, lo encaró, no logró buen éxito en la solución del mismo. No ha sido sino hasta la actualidad en que se cuenta con insecticidas y equipo que pueden usarse para luchar contra el insecto y, ya que día a día nuevos productos y nuevas técnicas van siendo encontradas es que el problema va siendo solucionado.

En asocio del Ing. R. Venegas, en el año de 1949 iniciamos algunos ensayos de control en la Meseta Central.

8 grupos de animales (vacas y bueyes) fueron formados, 4 de los cuales se bañaron cada 10 días y los cuatro restantes cada 3 semanas. Los tratamientos incluyeron tres repeticiones cada uno y constituían tratamientos a base de Clordano 50% WP, aplicado en tres diferentes concentraciones: 0.5%, 1%, 3% y 4%, usando para ello un spray-motor a 250 psi, en cantidad de 0.83 galones por animal. El ensayo cubrió el lapso de tiempo comprendido entre abril a agosto del año indicado.

Los resultados pueden ser observados en los gráficos (Fig. 2, 3), que se incluyen, los que muestran que (a) las diferentes concentraciones no dieron significación entre si, pero si entre ellas y el testigo, por lo que recomendamos usar la más baja proporción,

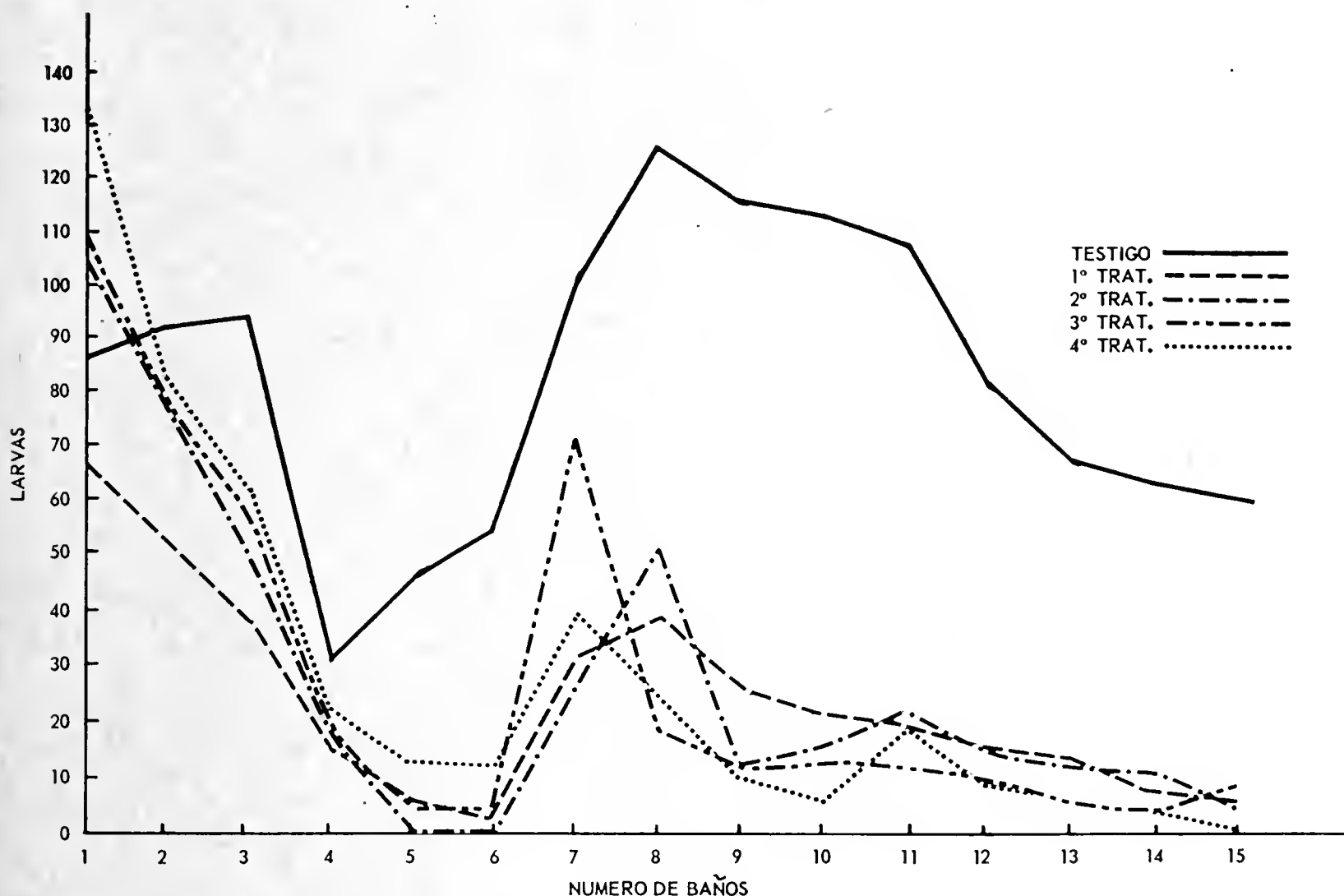


Fig. 2. Muestra la relación entre el testigo y los tratamientos. La columna vertical indica promedio de larvas por tratamiento, la horizontal numero de baños. Total 15 baños cada 10 días.

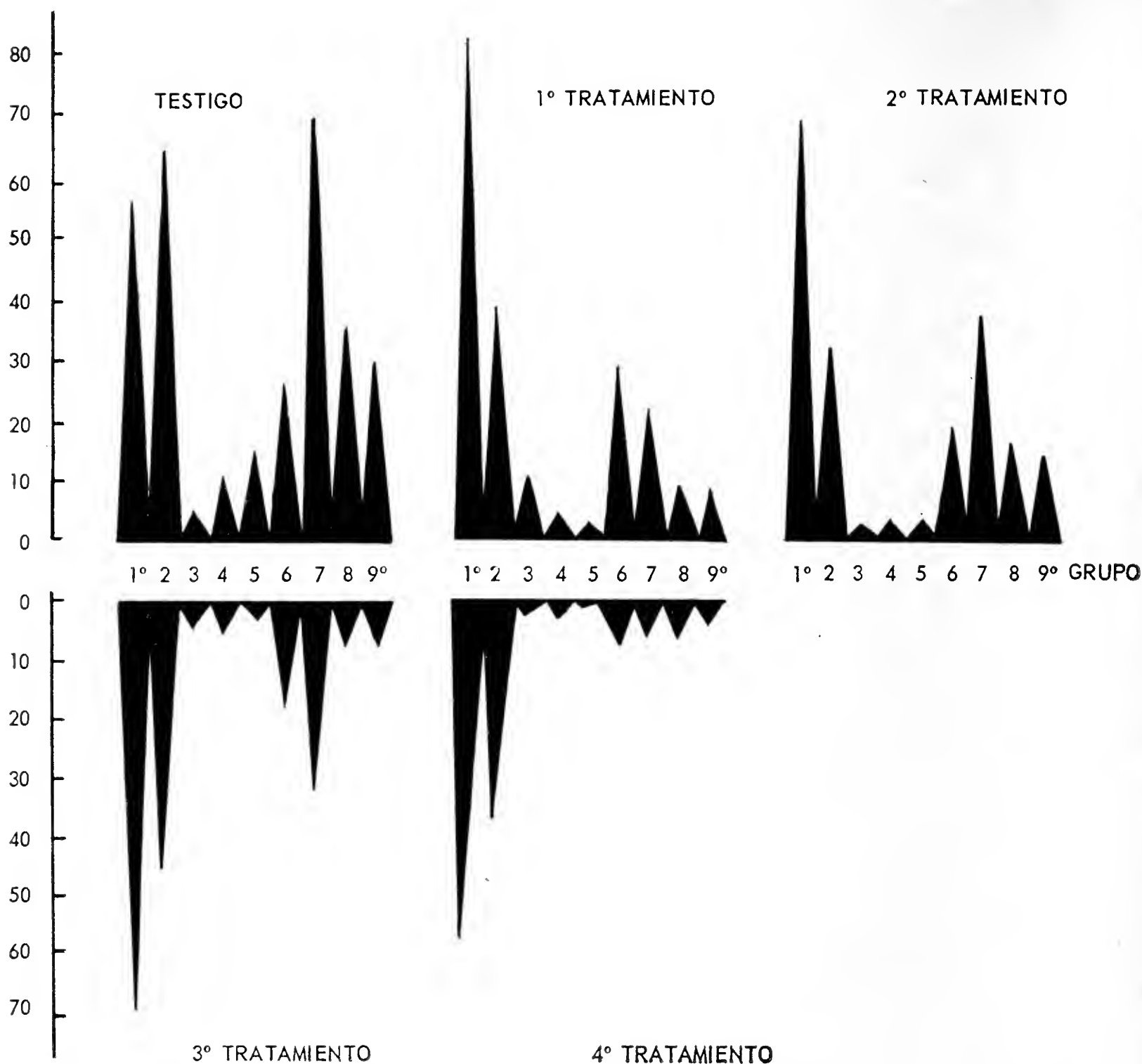


Fig. 3. Muestra la relación en población entre los grupos tratados y el testigo. Cada triángulo en cada tratamiento muestra un baño en el lote de animales (9 baños en cada grupo a intervalos de 22 días entre baño y baño).

cuando de polvos mojales se trate; (b) mayor diferencia hubo entre los tratamientos y testigo en la época seca; (c) en invierno el intervalo más recomendable es el de 10–15 días y en la seca de 3 semanas; (d) la población de larvas muestra fluctuaciones a lo largo del ensayo, pareciendo coincidir con el régimen de lluvias, puesto que baja en los meses secos (abril–mayo) y, sube al comenzar la lluvia, alcanzando el máximo en julio; e) la fluctuación pareciera tener relación también con la mayor o menor cantidad de vectores, pudiendo determinar que las larvas se enquistan en determinadas partes del cuerpo (partes altas o bajas) según la especie de portador dominante en la región (*Siphona irritans* o *Stomoxys calcitrans*).

Debemos mencionar que el insecticida en forma de polvo mojable en las concentraciones usadas, aun las más altas, no mostró síntomas de intoxicación.

El clordano en la actualidad ha sido bastante desplazado por otros insecticidas, en parte debido a la desventaja de ser absorbido y acumulado dentro del cuerpo de los animales. Pero entiendo que nuevos estudios del clordano aplicado a los animales están siendo realizados en USA, con el propósito de conocer si con el avance de los métodos de fabricación, esas desventajas han sido eliminadas o por lo menos reducidas.

Se ha afirmado en ciertas ocasiones que el Toxafeno es menos tóxico que el clordano cuando aplicado a los animales de sangre caliente, pero vemos que la dosis letal en ratas, según “The Comparative Insecticide, Fungicide, Herbicide Toxicities”<sup>2</sup> es la siguiente:

Clordano	457 mg/kg (ratas)	LD 50
Toxafeno	69 mg/kg	”
Lindano	125 mg/kg	”

<sup>2</sup>Assoc. of Food and Drug Officials of U.S. Vols. 15, #4 Oct. '51; Vol. 16, #1 Jan. '52 and Vol. 16, #2, April '52.

coincidiendo la misma relación en la dosis letal para terneras, según el Dr. Radeleff es:

Toxafeno	10 mg/kg
Clordano	75 mg/kg
Lindano	5 mg/kg.

Indicando que existe menor toxicidad en el clordano, lo que ha sido comprobado ya que habiendo ocurrido un número considerable de muertes por intoxicación de Toxafeno, no han ocurrido con clordano. Las anteriores consideraciones nos han inducido a pretender realizar nuevos ensayos para comparar estos dos materiales, siendo necesaria información sobre absorción y acumulación dentro del cuerpo.

Con la aparición de los clorinados y atendiendo la indicación de que el clordano es absorbido y acumulado en el cuerpo y liberado por medio de la leche, iniciamos en 1951, en asocio y asesorados por el Dr. Ernest W. Laake, pruebas con toxafeno emulsificable, en forma extensiva, acogiendo la serie de ensayos por él realizados en Brasil.

El producto fue aplicado en concentración de 0.5% en animales mayores de un año de edad, y a 0.25% en los menores de esa edad. También en este caso se usó igual presión y cantidad de líquido por animal. Dos intervalos de aplicación fueron probados: cada 2 y 3 semanas, de acuerdo al régimen de lluvias.

Los resultados obtenidos fueron sumamente satisfactorios, indicándonos la conveniencia de recomendarlo a los ganaderos, quienes lo acogieron con agrado, contando siempre con la ayuda que le proporcionó el Ministerio de Agricultura, en lo relacionado a asesoramiento, equipo e insecticidas a bajo costo, etc., proporcionándoles, en el caso de baños de inmersión, fórmulas seguras, con el fin de evitar intoxicaciones.

En algunas zonas, en que la población es sumamente alta (zona Norte de la Provincia de Alajuela y la del Atlántico, los animales a veces tienen entre 500 a 600 larvas enquistadas) nos atrevimos a probar un smear que contiene Lindano 3%, en aplicaciones locales muy reducidas, con el propósito de bajar con mayor rapidez la infestación, sobre todo en aquellos casos en que un número grande de larvas se concentran en ciertas partes del cuerpo del animal. Cuando bajaba la infestación, el smear era eliminado, continuando el tratamiento sólo con toxafeno. Los resultados fueron muy halagadores.

En ganado de carne ensayamos atomizaciones de lindano 0.03% WP, con el mismo propósito anterior, eliminándolo del tratamiento cuando bajaba la infestación. (La razón de no usar este producto a lo largo de todo el tratamiento se debe entre otras razones a su poco efecto residual).

Polvos mojables para uso en baños de inmersión debieron ser evaluados, entre ellos toxafeno 40% a 0.5%, con aparentes buenos resultados, siempre que se tuviera el cuidado de agitar la mezcla antes de cada baño, ó, en otros casos volver a pasar por el baño los animales que fueron primeramente bañados.

Mejores resultados fueron obtenidos cuando se usaron polvos mojables superfinos o supersuspendibles.

Igualmente, en ganado de carne fue empleado también el lindano 0.03%, con buenos resultados en los baños de inmersión.

Aunque algunos investigadores (A. A. Toledo y H. F. Sauer, Brasil, 1950) han trabajado en el control del dermatoparásito usando BHC, Toxafeno, Clordano y DDT en aplicaciones externas, también hicieron experimentos con BHC y clordano, aplicados oral e hipodérmicamente, encontrando que estos dos materiales eran los únicos que mostraban propiedades larvicidas. El isómero gama aplicado hipodérmicamente es lento en acción, pero ligeramente más rápido cuando se administró por vía oral. Los resultados no tienen todavía suficiente estudio como para ser aplicados prácticamente.

En el comercio se ofrecen ciertas fórmulas que no son conocidas en su constitución química, por lo que no nos atrevemos a usarlas a pesar de que se dice que inyectadas hipodérmicamente, eliminan la plaga en pocos meses y durante mucho tiempo.

## CONCLUSIONES

1. Para todos los materiales usados y en especial en los polvos mojables, es necesario que el animal tratado se seque completamente antes de entrar en contacto con la lluvia, se lo contrario la efectividad del material será reducida enormemente.

2. Es necesario, para el control del tórsalo, mantener sobre la piel del animal una película de insecticida, ya que los materiales usados, con excepción del smear de lindano 3%, no tienen efecto sobre las larvas enquistadas.

3. Los repastos han de mantenerse libres de malas hierbas, sobre todo si esta es alta y arbustiva. Los potreros y repastos muy enmontados son sitios ideales para el albergue de los adultos de tórsalo, y de ahí que el ganado en tales lugares es sumamente difícil de mantener limpio. En mi concepto la hierba alta tiene, sobre el insecticida, un efecto mecánico: su continuo rozar el cuerpo del animal, va eliminado, aunque lentamente, el insecticida.

4. Nuevos experimentos con clordano son necesarios en la actualidad, incluyendo algunos otros insecticidas que pueden ser eficientes.

5. El país debe organizar una campaña de control extensiva, haciendo uso de todos los medios a su disposición para lograr buenos resultados en corto tiempo, ya que en la actualidad se cuenta con insecticidas, equipo y personal, para llevarla a cabo con buen éxito.

Se cuenta con un servicio de extensión eficiente, el cual puede ser de gran ayuda en el desarrollo del programa. Existe también conocimiento del equipo que puede ser usado para la aplicación de los materiales. Para lograr éxito completo, leyes penando a los propietarios que tengan animales infestados deberán ser puestas en vigencia.

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# Factors which Attract *Aedes* Mosquitos to Humans

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## ABSTRACT

Studies on inanimate bodies previously reported, having shown that convective warmth, atmospheric humidity and carbon dioxide were attractive airborne factors, and that visual attractiveness increased with movement, contour and dullness of reflecting objects, experiments were performed with the human subject. Hands of warm-skinned Caucasian individuals were more attractive to *A. aegypti* than cool-skinned individuals, and an artificially cooled hand was much less attractive than a normal one. Hands of dark-skinned Caucasians were more attractive than those of light-skinned, but not as attractive as Orientals, which were surpassed by Negroes. Warmer skins were more attractive than cool, even if combined with a lighter skin hue, in Caucasian subjects. But the hands of individuals with low moisture output were more attractive than those of high moisture output, and a perspiring hand was less attractive than a normal one. Sweat from the armpits of Caucasian males was attractive to *A. aegypti*, but sweat from the forehead was not. Beef blood emits a volatile principle from the plasma that is attractive to *A. aegypti*. If carbon dioxide (and bicarbonate) is removed from the blood, it loses its attractiveness; and if  $\text{CO}_2$  is restored, the attractiveness partially reappears. No other compounds present in blood or sweat have proved to be attractive. When human clothing of 32 different hues were compared, their attractiveness to wild Canadian *Aedes* was in inverse ratio to their reflectivity of light between 475 (blue-green) and 625 (orange-red) millimicrons wave-length. Men in bright clothing attracted less mosquitoes to the face, as well as to the back, than men in dark clothing.

The realization that adult female mosquitos approach their animal host not as an act of volition, but as a token response to certain stimuli that the host introduces into the environment, has prompted the analytical method of studying the individual factors that might constitute stimuli. Our investigations into the responses of *Aedes aegypti* and of eastern Canadian *Aedes* in the field have been based on the choice of the mosquitos to approach either of two alternatives. The results obtained with inanimate objects such as robots in the field and spheres and olfactometers in the laboratory were communicated to the Ninth International Congress (Brown, 1952). The extension of this study, to include the human subject and substances such as sweat and blood, is reported to the present Congress.

Six factors could be separated and evaluated, namely the airborne factors of water vapour, carbon dioxide and convective heat, and the visual factors of movement, contour and reflectivity. Perhaps a brief recapitulation of these evaluations would be in order.

1. Moisture raises attractiveness 4 times. A moist air stream was 3–5 times as attractive as a dry one, a robot in wet clothing 2–4 times as attractive as one in dry, and a moistened sphere 5–7 times as attractive as its dry counterpart. The attractiveness decreased with increasing R.H. and decreasing temperature of the ambient air.

2. Carbon dioxide raises attractiveness 2 times. Addition of 10 per cent  $\text{CO}_2$  to an airstream more than doubled its attractiveness, and its emission at normal breathing rate increased the attractiveness of a robot by 50 per cent.

3. Convective heat raises attractiveness 3 times. Increase of the temperature of a spherical object by  $20^\circ\text{F}$ . increased its attractiveness by 2–5 times, and a robot heated to  $98^\circ\text{F}$ . was 3 times as attractive as an unheated one. The heat does not operate through radiation in the infra-red, but by convection through heat exchange to the air.

4. Movement raises attractiveness 2 times. Laboratory experiments with mice and inanimate models indicated that movement roughly doubles the attractiveness.

5. Contour raises attractiveness 1.6 times. An increase in black-white interface of checks increased attractiveness by 60%, and a mirroring effect increased it by 70%.

6. Reflectivity decreases attractiveness 5 times. A black surface attracted 5 times as many *A. aegypti* in the laboratory, and 4 to 10 times as many *Aedes* in the field, as a white one. Coloured cloths were attractive in inverse ratio to their brightness.

The airborne factors are at least equalled in effectiveness by the visual factors; a visible motionless animal in an airtight box attracted slightly more mosquitos than an invisible animal in a perforated box. But airborne factors are essential while the visual are not; Roth (1951) found that blinded mosquitos could locate a hand in a small cage, but that antennectomized ones did not. Thin-walled sensilla of both hair and peg type, present on the first and some of the other flagellar segments, allowed the perception of water vapour (Roth and Willis, 1952) and carbon dioxide (Willis and Roth, 1952).

The extension of this work since 1951 has been supported financially by the Defence Research Board of Canada, sometimes through the Canada Department of Agriculture. Workers associated with particular phases are: M. R. Smart with evaluations of human subjects, R. P. Thompson with studies on sweat, and L. Burgess with investigations on blood. Some of the results have been recently published (Thompson and Brown, 1955, Smart and Brown, 1956), and others are in press (Burgess and Brown).

The first project was to compare individual humans with each other, in order to ascertain the effects of skin temperature, skin hue and skin humidity on their attractiveness to female *Aedes aegypti*. The biophysical measurements were first made on the hand of the individual, and then the back of the hand was exposed to mosquitos in a large cage.

From a group of 150 young Caucasian males which were tested at rest by thermocouple, 7 cool-skinned individuals were selected with surface temperatures of 28.3° to 30.3°C. for comparison with 7 warm-skinned individuals, of similar complexion, but with surface temperatures of 30.6 to 33.2°C. When compared in pairs, the warm-skinned group attracted on the average 30% more mosquitos than the cool-skinned, the difference being highly significant.

When one of the hands of an individual was artificially cooled to 22°C. and compared to the other hand at its normal temperature of 30°C., the cooled hand was found to be one-quarter as attractive as the other, and its attractiveness increased as it returned to normal temperature. Similar results were obtained with laboratory rats by Kingscote and Francis (1954), who found that animals with a surface temperature of 30°C. were 4 times as attractive as those at 22°C. When one of the hands was heated to 38°C. and compared to the normal hand, it was less than half as attractive. On finally returning to normal temperature, it became just as attractive as normal despite the persistence of a red flush due to the treatment.

Next the factor of skin hue or reflectivity was investigated with the hands of young men. When 9 comparisons were made of darker-skinned Caucasians with light-skinned Caucasians of similar skin temperature, an average of 22% more landings occurred on the dark than on the light, and the difference showed high statistical significance. When dark but cool-skinned Caucasians were similarly compared with light but warm-skinned ones, the darker attracted 15 per cent more than the light despite the temperature difference, and the result was statistically significant.

Nine comparisons each were made of Negroes with fair Caucasians, Orientals with fair Caucasians, and Negroes with Orientals. The Negroes were found to attract 55% more mosquitos than the Orientals, which attracted 27% more than the Caucasians; when Negroes were compared directly with Caucasians, they attracted 60% more. The 27 individual comparisons of hands involved in these tests showed significant difference in all but 5 instances.

The factor of dermal transpiration of moisture was next investigated. From a group of 25 white males which were tested by enclosing the hands in polyethylene bags for 30 minutes, 7 moist-skinned subjects were selected with moisture outputs of 443 to 810 mg, and 7 dry-skinned subjects with moisture outputs of 198 to 338 mg. These individuals were tested again a few months later and found to have the same moisture characteristics. From 26 paired comparisons between the members of either class, it became evident that it was the dry-skinned individuals that attracted more mosquitos, inducing on the average 48% more landings, and the difference was highly significant. Moreover, the greater the difference in moisture output between the pair tested, the greater was the differential attractiveness of the individual with the dry skin; the correlation coefficient for this relationship proved to be 0.72, which is of high statistical significance.

In a second set of experiments to pursue this unexpected result, one hand was induced to perspire by vasodilation and compared with the other hand in normal condition. The

right arm was heated in dry air at 140°F. for 15 minutes, and the hand was then tested when its surface temperature had returned to normal. A total of 10 individuals provided test material, and the increase in transpiration induced ranged from 2 to 102%, averaging 39%. Their normal hands were found to attract on the average 34% more mosquitos than the treated hands. Moreover the greater the difference in moisture output between the hands, the greater was the differential attractiveness of the drier hand, the correlation coefficient being 0.75.

The results of these studies on human subjects, then, agree with those made on inanimate objects with regard to temperature and reflectivity, but they do not in the case of moisture. The source of moisture from the human skin is not only transpiration from the dilated capillaries but also the secretion of sweat from the sudoriferous glands particularly abundant on the hands. As will be seen below, sudor is no more attractive than water. It is possible that a high moisture emission by individuals is associated with output of less attractive odours, but we have no evidence on this point. But there is evidence that very high moisture in an air stream will reduce the response of mosquitos to CO<sub>2</sub> (Brown, Sarkaria and Thompson, 1951) and to body odours of host animals (Laarman, 1955).

It is of interest to note that one of the 14 subjects studied had an unusually high moisture output and was unusually unattractive to mosquitos. In appearance and name he resembled a Caucasian, but his uniqueness in these experiments prompted enquiries which revealed that both his parents were Hungarian, a race of Asiatic origin.

In these studies on humans, caution should be exercised against assuming that each individual has a characteristic attractiveness. In the experiments of Ribbands (1949) with 3 Africans exposed to *Anopheles gambiae* and *A. funestus* in identical huts, one individual would be much more attractive than the others for sequences of 3 to 8 days. But any one of the three could be the attractive one, so that the total catch of mosquitos for the entire period of study was approximately the same for each man.

The next line of attack was to investigate the attractiveness of human sweat. This is of two kinds; sudor, which is produced by simple glands over the general body surface, especially the forehead and the palms of the hands; and sebum, produced by complex glands particularly in the armpits and inguinal regions. Sudor is an aqueous solution of inorganic salts such as sodium chloride, together with the non-colloidal organic compounds present in blood; sebum contains lower fatty acids, esters of higher alcohols, cholesterol, and some albumin. Early experiments by Howlett (1910) and Crumb (1922) failed to show any attraction in sweat for adult *Aedes* or *Culex*. Rudolfs (1922) could not detect any attractiveness in either sudor or sebum for *Aedes sollicitans* and *A. cantator*. Reuter (1936) found that neither type of sweat attracted *Anopheles maculipennis atroparvus*. More recently Parker (1948) has found armpit sweat to be more attractive than water alone to *Aedes aegypti*.

Our first experiments were performed in the field with robots; clothing soaked with sweat from the armpits and general body surface of Caucasian males was twice as attractive as equally moist clothing to *Aedes punctor* and *A. communis*. But when this experiment was repeated with cloth coverings of spheres in a greenhouse, *Aedes aegypti* betrayed no preference for armpit sweat.

Experiments were then performed with the olfactometer emitting into a very large cage, as described by Brown, Sarkaria and Thompson (1951), and armpit sweat was tested after dilution with water to 30, 3 and 0.3 mg per cc. On comparison with water vapour, the lowest dilution showed no significant attraction; but the other two dilutions were significantly attractive, and at 3 mg per cc the sweat consistently attracted about 25 per cent more *Aedes aegypti* than water alone. Armpit sweat is rich in sebum. When sudor alone as obtained from the forehead was similarly tested, it showed no significant attraction at any of the 3 concentrations.

Van Thiel (1937) had observed cursorily that sweating greatly increased his own attractiveness to *Anopheles m. atroparvus*, and suggested that CO<sub>2</sub>, which he had found very attractive to this species and *A. aegypti*, was the principal operative factor in sweat. Our own experiments on this matter were inconclusive. CO<sub>2</sub>-saturated water in the clothing of robots proved unattractive in the field, where sweat had proved attractive as already mentioned. On the other hand, CO<sub>2</sub>-saturated water in cloth coverings of spheres made them more attractive to *Aedes aegypti*, while as already noted sweat had not showed attraction. An aqueous solution of CO<sub>2</sub> and bicarbonate in the concentrations found in



sweat was not significantly more attractive than water in the olfactometer. Addition of excess  $\text{CO}_2$ , or alternatively the removal of bicarbonate by  $\text{BaCl}_2$ , failed to change the level of attractiveness of armpit sweat.

These findings render it unlikely that  $\text{CO}_2$  is responsible for much of the attractiveness of human sweat in the olfactometer in a large cage. In this it probably resembles the picture obtained from a study of the "insensible perspiration" of resting men and non-sweating animals. Willis (1948) was able to demonstrate in an olfactometer that the vapour from the arm and hand, when compared with moist air, was nearly twice as attractive to *Aedes aegypti* and almost 3 times to *Anopheles quadrimaculatus*; yet he was unable to find any concentration of  $\text{CO}_2$  which could give any attractiveness to these species in this small-cage olfactometer. Subsequently Willis and Roth (1952) showed that  $\text{CO}_2$  was attractive in a large-cage olfactometer similar to the one we used, where it was not so in a small cage. Laarman (1955), using an olfactometer in which the cage was not enclosed, found that the vapour obtained from an incarcerated rabbit was even more attractive to *Anopheles m. atroparvus* than the human arm. When  $\text{CO}_2$  was removed from this rabbit vapour, its "attractivity index" fell from 92.4% down to 87.6%, as if this gas was responsible for a certain fraction of the rabbit's attractiveness, but certainly not all; for if  $\text{CO}_2$  was added in excess to the control air stream, the rabbit odour was still twice as attractive as the control.

It is probable that the role of  $\text{CO}_2$  in human attractiveness varies greatly with other conditions. Olfactometer experiments have shown both for *Aedes aegypti* (Brown, Sarkaria and Thompson, 1951) and for *Anopheles m. atroparvus* (Laarman, 1955), that  $\text{CO}_2$  is unattractive in a humid air-stream in concentrations which are attractive in a dry one. The general activating effect of  $\text{CO}_2$  was first noted by Rudolfs (1922), and subsequently by Willis (1948) and De Long (1949) who considered it to be an activating rather than an orienting stimulus; Laarman (1955) concedes its activating characteristic and its role in potentiating the truly orienting stimuli, but can envisage situations where the activity due to  $\text{CO}_2$  can result in orientation. He found that the effect of the human breath on *Anopheles m. atroparvus*, which showed an attractivity index of 90% in his olfactometer, was virtually entirely due to the  $\text{CO}_2$  in it; for if  $\text{CO}_2$  was added to the control air-stream, the attractiveness of the breadth disappeared. Evidently the  $\text{CO}_2$  in the expired air is far more important in attracting mosquitos than the  $\text{CO}_2$  in the insensible perspiration, the concentration being 3.5 times greater in the former case. The relative importance of these two types of body exudation has been evaluated for the human subject by Mer, Birnbaum and Aioub (1947), using *Anopheles m. elutus*; by removing the expired air they made the following discoveries. If the mosquitos are above the man it does not matter whether the breadth is present or not, convection currents carrying his skin exudation upward make him highly attractive. If the mosquitos are alongside the man, only his directed breath can make him attractive; if his body exudation is directed with a fan, a lesser attraction can be achieved.

The search for the attractive elements in sweat other than  $\text{CO}_2$  proved fruitless in our experiments. Acidification of the sweat to release the organic acids in higher concentration only served to make it repellent; making it alkaline did not alter its attractiveness. None of the fatty acids tested by other workers or by us have proved attractive, and these include formic, acetic, propionic, lactic, butyric, caproic, valeric and oleic acids (Rudolfs 1922; Reuter, 1936; De Long 1949; Brown, Sarkaria and Thompson, 1951); the only exception was that Rudolfs (1922) found benzoic acid attractive. Of the nitrogenous bases, ammonia, trimethylamine, indole and skatole have been found unattractive (Reuter, 1936; Brown, Sarkaria and Thompson, 1951), though again it was Rudolfs (1922) who found dilute ammonia to be attractive. Rudolfs (1922) had found that certain amino-acids, which could be expected to arise from hydrolysis of albumin in the sebum, were attractive to *Aedes sollicitans* and *A. cantatar*; these were phenylalanine, alanine, aspartic acid, and (to a lesser extent) cystine. But Reuter (1936) found that none of these amino-acids were attractive to *Anopheles m. atroparvus*. Rudolfs (1922), however, found that peptone was highly attractive to his *Aedes*.

That there is some attractiveness in the accumulation of body exudations is indicated by the discovery of Haddow (1942) that *Anopheles gambiae* and *A. funestus* are more attracted to unwashed African children than to washed, and that the presence of dirty clothing attracts more of these mosquitos into huts.



We next proceeded to investigate the attractiveness of blood. Early experiments by Howlett (1910) and by Rudolfs (1922) had indicated that exposed blood as shed was not attractive to *Aedes* mosquitos. Eventually Reuter (1936) found that defibrinated pig blood taken up on filter paper and exposed on a heated "artificial arm" was occasionally slightly attractive to *Anopheles m. atroparvus* in a small cage. Van Thiel and Weurman (1947), studying this species in the field, discovered that such blood exposed on cloth increased the attractiveness of a heated box by 8 times as compared with water alone. More recently, Schaerffenberg and Kupka (1951) found that the addition of fresh blood to fly papers increased their catches of *Culex pipiens* and *Anopheles maculipennis* by 10 times.

In our experiments, it was discovered that heparinized beef blood exposed on filter paper was 3 times more attractive to *Aedes aegypti* than the washed corpuscles, and that the plasma was about 5 times as attractive as water. When an air-stream was passed through the blood and tested in an olfactometer, it proved significantly more attractive than water vapour. Laarman (1955) also found that the vapour from citrated rabbit blood was over twice as attractive in an olfactometer as that from physiological saline, to *Anopheles m. atroparvus*.

When the vapour from beef blood was passed through a solution of  $\text{Ba}(\text{OH})_2$ , which removes  $\text{CO}_2$ , the attractiveness to *Aedes aegypti* disappeared. When the blood itself was treated with a vacuum to remove the volatile substances, it still remained attractive; after treatment with  $\text{BaCl}_2$  to remove the bicarbonates, it was still attractive; but when it was treated with both vacuum and  $\text{BaCl}_2$ , thus entirely removing its ability to evolve  $\text{CO}_2$ , its attractiveness finally disappeared. It was also found that the attractiveness was completely removed by desiccation and was not restored by remoistening.

Since these experiments showed that only when the  $\text{CO}_2$ -producing ability was completely removed was the attractiveness of blood completely abolished, the effect of restoring  $\text{CO}_2$  to the blood was investigated. When an amount was restored which made the  $\text{CO}_2$  evolution equivalent to that of whole blood, the attractiveness was significantly increased; but it reached only half the level of attractiveness shown by the original whole blood. It would therefore appear from these experiments that  $\text{CO}_2$  is responsible for only a certain fraction (perhaps about a quarter) of the attractiveness of beef blood to *Aedes aegypti* in the laboratory. In the open-air experiments with *Anopheles m. atroparvus*, van Thiel and Weurman (1947) found that, just as the addition of pig blood increased the attractiveness of a  $\text{CO}_2$ -containing system by 7 times, so the addition of  $\text{CO}_2$  to a blood-containing system increased its attractiveness by 7 times also.

What the attractive elements in blood are, other than  $\text{CO}_2$ , remains unknown. We were unable to demonstrate an attractant in the ether extract of blood. Rudolfs (1922) had reported that haemoglobin was attractive to his *Aedes* in test-tubes; but since Reuter (1936) found that this compound, which has no appreciable volatility whatsoever, had no attraction for *Anopheles*, it is possible that Rudolf's preparation of haemoglobin had biologically active contaminants. A most promising development appeared in the report by Schaerffenberg and Kupka (1951) of a substance obtained from blood that was highly attractive to *Culex pipiens*. This Blutduftstoffe is described as a colourless solution with a sweet aromatic odour somewhat similar to that of blood, that is 5 times more attractive than water even when diluted 2000 times. Unfortunately the published report does not specify the source of the blood or describe the method of preparation.

It was van Thiel (1937) who first made the general statement that the attractiveness of a warmblooded host to mosquitos could be attributed to the  $\text{CO}_2$ , moisture and warmth it emitted, possibly reinforced by odorous substances from the blood. It has been postulated by Schaerffenberg and Kupka (1951), and the experiments of Laarman (1955) have supported this postulation, that the diffusion of odorous substances from the epithelia of lung, skin and mouth is an important element in the attractiveness of the host. It has been pointed out by Laarman that such specialized substances offer the only means of explaining host selection in olfactometers or appareils de choix, where *Anopheles m. atroparvus* will prefer, on olfactory stimuli only, pig to man in the ratio of 12 to 1, while *elutus* and *labbranchiae* prefer man to pig more than 2 to 1 (van Thiel 1939); or where air-streams containing rabbit odour are preferred to human arm odour, and rabbit blood gives much greater vapour attractiveness than beef blood, to the same species *Anopheles m. atroparvus* (Laarman, 1955). Our experiments with *Aedes aegypti* have assessed the roles of  $\text{CO}_2$ , moisture and surface heat in

determining the relative attractiveness of individuals, and have roughly evaluated the importance of CO<sub>2</sub> as a factor in sweat and in blood; but they have nothing to offer on the nature of the specific olfactory attractants beyond giving evidence for their existence.

Since 1951 we have been able to advance the knowledge of visual attractiveness to mosquitos with respect to reflectivity and hence to colour. The attractiveness of 32 different cloths to eastern Canadian *Aedes* was assessed both on robots and on man, and their ranking proved to be similar in either case, the dark reds, dark blues and blacks being preferred to the yellows, light greens and whites (Brown, 1954). The reflectivity of these cloths was determined physically for the different wavelengths of visible light, and also in the ultra-violet and infra-red ranges. No correlation whatever was found between attractiveness and reflectivity in either the infra-red or ultra-violet range of wavelength; but a definite negative correlation existed within the visible range, in that lower the reflectivity of visible light by the cloth, the greater the attractiveness it offered to the mosquitos. Three series of 8, 7 and 5 cloths respectively, identical in texture but differing only in colour, offered an opportunity to delimit the negative correlation more closely. It was found to exist only in the range between 475 and 625 millimicrons wavelength, i.e. light between the blue-green and the orange. No correlation existed at longer wavelengths in the red or at shorter wavelengths in the blue or violet, as if these *Aedes* mosquitos were photosensitive only to the middle half of the humanly visible spectrum.

It therefore becomes evident that the more the clothing reflects in wavelengths between blue-green and orange, the less attractive it will be. The question remained whether unattractive clothing would decrease the attack on the face and hands of the wearer, or increase it by discouraging the mosquitos from landing on the garment itself. This was put to the test in the field, by comparing a green and a white suit with a blue and a black one (Brown, 1955). It was found that with the unattractive clothing, the ratio of mosquito attack on the exposed face to that of the clothed back was indeed 20% higher than with the attractive clothing. But on the average 33% fewer mosquitos landed on the unattractive clothing, and the number of landings on the face in fact averaged 16% fewer with the unattractive than with the attractive clothing.

The results of our somewhat utilitarian studies, therefore, may be recapitulated in fairly concrete terms, as far as *Aedes* mosquitos are concerned. Cooler, paler moister-skinned individuals may be expected to be less attractive than warmer, darker, drier-skinned individuals. Vasodilation, heightened surface temperature and sweat all increase attractiveness, and so does movement. On the other hand, light-hued or luminescent clothing, particularly white, yellow or green, decrease the attractiveness of the wearer to these mosquitos. All that a cool moist-skinned pale motionless man in a white suit has to do to ensure freedom from mosquito attack is, in the words of Allen Rankin of *La Revue Moderne*, to "stop breathing".

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### DISCUSSION

H. I. SCUDDER. At the Hooper Foundation and Public Health Service Laboratory in Bakersfield, California, certain domestic chickens were found to be more attractive to mosquitoes from a distance, yet failed in acceptance as blood hosts, while other chickens, not apparently attractive, were fed upon readily by mosquitoes in their immediate vicinity. The experiment was with *Culex tarsalis* by Reeves, Ballamy, Hayes, and Brookman.

R. M. GORDON. Muirhead-Thomson has suggested that in the West Indies and in Africa native infants were consistently less attractive to anophelines than were older children and adults when exposed under similar conditions. Has Dr. Brown observed this "age factor" in the case of *Aedes*?





# Sensory Organs of Blood-Feeding Diptera

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## ABSTRACT<sup>1</sup>

Intricate sensory equipment is required for seeking and finding a vertebrate host, and feeding in spite of interference by the host. Also, other sensory needs of mating and oviposition must be satisfied. The sensory organs are of many types, some innervated by as many as eight neurons. Some chemoreceptors specifically serve for "near-orientation". "Contact" chemoreceptors occur as highly specialized tubular hairs on the labellar fringes of the mouthparts. Flow-measuring sensory organs are minute hairs projecting into the lumen of the blood-sucking channel of the proboscis. Specialized tactile receptors for proprioception occur along and at the ends of the pseudotracheal canals of the labellar lobes. Minute, unsheathed, and freely branching nerve plexi in the integument have specialized endings suggested to be thermoreceptors. Functional interpretation of such sensory organs can assist in studies of behavior and in research on repellents and attractants. Studies of insect sensory organs may add materially to our general knowledge and interpretation of those animal sensory structures now poorly understood.

## DISCUSSION

V. G. DETHIER. In the blow fly, two separate nerve strands continue to the tip of the gustatory hairs of the labellum.

H. I. SCUDDER. In *Tabanus* labellar gustatory hairs, the terminal nerve fibers eventually fuse roughly halfway toward the apex of the hairs, so that toward the tip only a single fiber exists. This fiber "twigs" off along its course toward the tip, rather than remaining unchanged, or as two separate strands, all the way to the tip.

<sup>1</sup>Complete paper to be submitted to *Acta Tropica*.



# Feeding Techniques for Bloodsucking Arthropods

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## ABSTRACT

An apparatus and a technique are described that were utilized for the *in vitro* feeding of fleas (*Xenopsylla cheopis*), mosquitoes (*Aedes aegypti*), and ticks (*Ornithodoros moubata*). The apparatus consists of (1) a thermostatically controlled water bath, (2) a container to hold blood, and (3) a feeding cup with a chamber to hold the arthropods and a membrane through which they can feed. The following conditions were found to be optimum for feeding fleas, ticks, and mosquitoes through membranes: (1) freshly drawn or thawed, heparinized beef, rat, out-dated whole human, and guinea pig bloods; (2) a blood temperature of 34°C.; (3) an ambient temperature of from 19–30°C.; (4) a very thin animal-derived membrane, and (5) a feeding period of 60 minutes. Of the 29 membranes screened, 23 were new for *in vitro* feeding. Some of the new membranes were highly satisfactory for membrane feeding; many of the others were insufficiently tested and should be re-screened. The best membranes for fleas were hamster skin (97% fed), rat skin (95% fed), Baudruche Transparent (94% fed), Silverlight (85% fed), and rabbit skin (82% fed). Silverlight was found best for mosquitoes (75% fed) and rabbit skin for ticks (97% fed). This apparatus and technique should have a wide range of applications as they could be valuable aids in basic entomological research on such problems as the transmission of bacterial, viral, and protozoan micro-organisms; physiology and nutrition; the chemotherapeutic control of ectoparasites; and behavior.

## INTRODUCTION

Probably the first successful feeding of arthropods through membranes was accomplished in 1912 by Rodhain and his coworkers who successfully fed tsetse flies on blood through rat skin membranes during their studies on African trypanosomiasis. Since then a multitude of techniques and membranes have been used for the *in vitro* feeding of haematophagous arthropods.

Studies relating to the transmission of leishmaniasis (Adler and Theodor, 1927a, 1927b, 1931, 1939), toxoplasmosis (Woke, *et al.*, 1953), avirulent plague (Kartman, 1954; Quan, *et al.*, 1954; Wheeler, *et al.*, 1956), tularemia (Price, 1954), and epidemic typhus (Fuller, *et al.*, 1949; Fuller, 1953) have all been successfully carried out with the aid of membrane feeding. Yoeli (1938) was able to infect *Anopheles elutus* with *Plasmodium falciparum* by feeding the mosquitoes through rabbit skin membranes on infected blood. Bishop and Gilchrist (1946) were able to infect *Aedes aegypti* with *Plasmodium gallinaceum* when these mosquitoes were fed on infected blood through a chicken skin membrane, and the mosquitoes so infected were able to inject viable sporozoites through a membrane into clean blood.

Rivnay (1930) and De Meillon and Golberg (1947) used membrane feeding to study the tropisms of *Cimex lectularius* and the nutritional requirements of this insect, respectively. Nicolle and Lwoff (1942) used membrane feeding to study the nutritional requirements of *Triatoma infestans*. Membrane feeding was used by De Meillon, *et al.* (1948) for the rapid screening of drugs for possible use in the chemotherapeutic control of ectoparasites. These studies and the details listed in Table I give but just a glimpse of the wide variety of entomological work in which *in vitro* feeding with membranes can be valuably, and oftentimes indispensably, utilized.

Despite the fact that the literature on membrane feeding is extensive already, there are still better membranes, techniques, and pieces of equipment to be found for obtaining higher and more certain feeding rates. This paper describes a number of new membranes, some apparatus that has been used for feeding fleas (*Xenopsylla cheopis*), ticks (*Ornithodoros moubata*), and mosquitoes (*Aedes aegypti*) with a high degree of efficiency, and results of experiments with the membranes and apparatus.

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TABLE I. Membranes Utilized by Various Investigators.

Membranes	Arthropods	Investigators
Fresh mouse skin	<i>Pediculus humanus corpori</i>	Buxton, 1946
Mouse skin	<i>Cimex lectularius</i>	DeMeillon and Golberg, 1947
Mouse skin	<i>Rhodinus prolixus</i> <i>C. lectularius</i>	DeMeillon, Thorp and Hardy, 1948
Newborn white mouse skin	<i>Bdellonyssus bacoti</i>	Macdonald and Scott, 1952
Three-week-old mouse skin	<i>Melophagus ovinus</i> <i>Haematopinus suis</i> <i>H. eurysternus</i> <i>Linognathus vituli</i> <i>L. setosus</i>	Nelson, 1955
Mouse epidermis	<i>Pediculus vestimenti</i>	Puchta, 1955
Rat skin (clipped)	<i>Aedes albopictus</i> <i>Aedes aegypti</i> <i>Culex pipiens</i>	Jackowski, 1954
Juvenile albino rat skin	<i>Xenopsylla cheopis</i>	Kartman, 1954
Adult albino rat skin	" "	Kartman, 1954
Newborn cotton rat skin	<i>B. bacoti</i>	Macdonald Scott, 1952
Rat skin	<i>X. cheopis</i>	Quan, Kartman and McManus, 1954
Rat skin	" " <i>X. vexabilis hawaiiensis</i>	Kartman, Quan and McManus, 1956
Rat skin	<i>Glossina palpalis</i>	Rodhain, et al., 1912
Albino rat skin	<i>X. cheopis</i>	Wheeler, et al., 1956
Week-old rat skins	<i>A. aegypti</i>	Woke, 1937
Juvenile guinea pig skin	<i>X. cheopis</i>	Kartman, 1954
Adult guinea pig skin	<i>X. cheopis</i>	Kartman, 1954
Rabbit skin	<i>Phlebotomus papatassi</i>	Adler and Theodor, 1927a
Rabbit skin	" "	Adler and Theodor, 1927b
Rabbit skin	" "	Adler and Theodor, 1931
Rabbit skin	" "	Adler and Theodor, 1939
Rabbit ear skin	<i>Anopheles elutus</i>	Yoeli, 1938
Chicken skin	<i>C. lectularius</i>	Andre, 1912
One- to three-week-old chick skin	<i>A. aegypti</i>	Bishop and Gilchrist, 1944
Two- to three-week-old chick skin	" "	Bishop and Gilchrist, 1946
Chicken skin	<i>P. humanus corporis</i>	Fuller, 1953
One- to seven-day-old chick skin	" " "	Fuller, Murray and Synder, 1949
Dehydrated chick skin	<i>A. aegypti</i>	Greenberg, 1951
Chicken skin	" " <i>A. albopictus</i> <i>C. pipiens</i>	Jackowski, 1954
Four-week-old chick skin	<i>Melophagus ovinus</i> <i>H. suis</i> <i>H. eurysternus</i> <i>L. vituli</i> <i>L. setosus</i>	Nelson, 1955



TABLE I. (Continued)

Membranes	Arthropods	Investigators
Chicken skin	<i>P. humanus corporis</i>	Price, 1954
Chicken skin	<i>Anopheles</i> spp. <i>Aedes</i> spp. <i>C. pipiens</i>	Trembley, 1952
Bat skin	<i>Aedes calopus</i>	Gordon, 1922
Week-old duckling skin	<i>B. bacoti</i>	Macdonald and Scott, 1952
Two-day-old turkey poult skin	<i>M. ovinus</i> <i>H. suis</i> <i>H. eurysternus</i> <i>L. vituli</i> <i>L. setosus</i>	Nelson, 1955
Animal membrane	<i>Pseudolynchia canariensis</i>	Prouty and Coatney, 1933
Animal skins	<i>Aedes scutellaris</i>	Sen, 1917
Animal membrane	<i>Anopheles elutus</i>	Yoeli and Mer, 1938
Human cadaver skin	<i>P. humanus humanus</i>	Pshenichnev, 1943
Human cadaver skin	" " "	Raikher, 1943
Human skin prepared for split thickness grafting	<i>B. bacoti</i>	Macdonald and Scott, 1952
Baudruche capping membrane	<i>A. aegypti</i>	Greenberg, 1949, 1951
Baudruche capping membrane	" "	Shambaugh, 1954
Baudruche capping membrane	<i>Anopheles</i> spp.	Trembley, 1952
Animal mesentery bag "fish skins"	<i>Aedes dorsalis</i> <i>A. nigromaculis</i> <i>A. campestris</i> <i>A. fitchii</i> <i>A. vexans</i> <i>A. stimulans</i> <i>A. increpitus</i> <i>A. cinereus</i> <i>A. niphadopsis</i> <i>Anopheles maculipennis</i> <i>Theobaldia inornata</i> <i>T. incidens</i> <i>Culex tarsalis</i> <i>C. salinarius</i>	Knowlton and Rowe, 1935
Sheep mesentery	<i>A. aegypti</i> <i>A. albopictus</i> <i>C. pipiens</i>	Jackowski, 1954
Cotton rat intestine	<i>B. bacoti</i>	Macdonald and Scott, 1952
Mouse intestine	" "	Macdonald and Scott, 1952
Fish intestine	<i>C. lectularius</i>	Rivnay, 1930
Rabbit intestine	" "	Rivnay, 1930
"Bleb"	<i>P. humanus corporis</i>	Synder and Wheeler, 1945
Shell membrane	<i>A. aegypti</i>	Davies and Yoshpe-Purer, 1954
Egg shell membrane	Mosquito Stable fly	Ferris and Hanson, 1952
Shell membrane	<i>A. aegypti</i>	Goldwasser and Davies, 1953
Egg shell membrane	" "	Haas and Ewing, 1945

TABLE I. (Continued)

Membranes	Arthropods	Investigators
Egg shell membrane	<i>C. lectularius</i>	Rivnay, 1930
Baby chick cecum	<i>B. bacoti</i>	Macdonald and Scott, 1952
Newborn mouse bladder	" "	Macdonald and Scott, 1952
Pig bladder	<i>A. aegypti</i> <i>A. albopictus</i> <i>C. pipiens</i>	Jackowski, 1954
Hog-gut sausage casings	<i>A. aegypti</i>	Eyles, 1952
Hog-gut sausage casings	" "	Kartman, 1953
"Visking" sausage casing	<i>B. bacoti</i>	Macdonald and Scott, 1952
Sausage skins	Lice	Moore and Hirschfelder, 1919
Albumin cloth	<i>A. scutellaris</i>	Sen, 1917
Boiled egg membrane	<i>A. aegypti</i> <i>A. albopictus</i> <i>C. pipiens</i>	Jackowski, 1954
Latex membranes	<i>C. lectularius</i> <i>R. prolixus</i>	DeMeillon, Thorp and Hardy, 1948
Latex membranes	<i>A. aegypti</i> <i>A. albopictus</i> <i>C. pipiens</i>	Jackowski, 1954
Latex membranes	Reduviids	Nicolle, 1941
Latex membranes	Reduviids	Nicolle, 1944
Latex membranes	<i>Triatoma infestans</i>	Nicolle and Lwoff, 1942
Latex membranes	<i>Triatoma infestans</i> <i>Rhodnius prolixus</i>	Nicolle and Mathis, 1941a
Latex membranes	<i>Triatoma infestans</i>	Nicolle and Mathis, 1941b
Cellophane	<i>P. humanus corporis</i>	Haddon, 1956
Cellophane	<i>A. aegypti</i> <i>A. albopictus</i> <i>C. pipiens</i>	Jackowski, 1954
Cellophane	<i>B. bacoti</i>	Macdonald and Scott, 1952
Cellophane	<i>Ixodes ricinus</i> <i>Hyalomma marginatus brionicum</i>	Totze, 1933
Cellophane	<i>C. pipiens</i> <i>Stomoxys calcitrans</i> <i>Pulex irritans</i> <i>Ctenocephalides canis</i> <i>Ceratophyllus fasciatus</i>	Totze, 1934
Soaked gelatin	<i>Aedes scutellaris</i>	Sen, 1917
Tough cloth	" "	Sen, 1917
Tender Plantain shoots	" "	Sen, 1917
Toy balloons	" "	Sen, 1917
Parchment weighing papers	<i>A. aegypti</i> <i>A. albopictus</i> <i>C. pipiens</i>	Jackowski, 1954

TABLE I. (Continued)

Membranes	Arthropods	Investigators
Collodion	<i>A. aegypti</i> <i>A. albopictus</i> <i>C. pipiens</i>	Jackowski, 1954
Onion skin	<i>A. aegypti</i> <i>A. albopictus</i> <i>C. pipiens</i>	Jackowski, 1954
Saran	<i>P. humanus corporis</i>	Haddon, 1956a
Mylar	" " "	Haddon, 1956a
Polyethylene	" " "	Haddon, 1956a
Parafilm	" " "	Haddon, 1956a
Gutta percha	" " "	Haddon, 1956a
Gutta percha	" " "	Haddon, 1956b
Silk bolting cloth	<i>Bdellonyssus bursa</i> <i>B. bacoti</i> <i>B. sylviarum</i> <i>Dermanyssus gallinae</i>	Cross, 1954
Organdi	<i>Anopheles</i> spp.	Jepson, <i>et al.</i> , 1947

MATERIALS AND METHODS

MEMBRANE FEEDING APPARATUS

During the early experimental work fleas and ticks were fed in membrane cups that were altered two-ounce metal ointment cans, 2½ inches in diameter and ¾ths of an inch in height. A 2½-inch hole was cut in the bottom of the can and a membrane secured over it with a double strand of #24 gauge copper wire. To prevent blood leakage 1/2-inch waterproof plastic tape was wrapped around the can over the wire and membrane edge. A 1 7/8-inch hole was cut in the ointment can lid and a piece of 80 mesh stainless steel screen cloth was soldered to the inside of the lid, covering the hole. During feeding these membrane cups were floated on blood contained in a large, flat enameled ware pan which rested on two metal rods in a large, open water bath.

Though the above membrane apparatus was used for a considerable period of time it was not wholly satisfactory. When the cups were allowed to float free on the blood surface they were difficult to handle, blood often leaked into them and arthropods could escape if small unsoldered holes were left around the screening. If the lids were soldered too heavily the cups sank to the bottom of the blood and drowned the arthropods. Furthermore, there is a possibility that the heat rising from the open blood and water baths raised the ambient temperatures sufficiently to hinder feeding by the arthropods.

Thus, after a time, a new membrane feeding apparatus (Fig. 1) was made, and since then this basic design has been used exclusively. The essential parts of the apparatus, almost entirely of plexiglas, are membrane feeding cups and adaptor rings, a blood chamber and a closed water bath (Figs. 2 and 3). The water bath is heated by a thermostatically controlled 25-watt aquarium immersion heater<sup>2</sup>. The membranes are affixed to the plexiglas cups with plexiglas rings (Fig. 4).

For screening large numbers of membranes and different bloods simultaneously, a plexiglas apparatus approximately four times the size of the single unit one was built (Figs. 5 and 6). It has four blood chambers. Any of the three assembled feeding cups of the single unit apparatus can be used with the four chamber apparatus.

The original mosquito membrane feeding cup was a 6½ inch-high, 2 3/8-inch diameter, #20 mesh, copper-wire-screen-cloth cylinder. The bottom of an ointment can membrane cup fitted into the bottom opening of the cylinder and the top of an ointment can was

<sup>2</sup>Distributed by Eugene Danner Company, Brooklyn, New York.

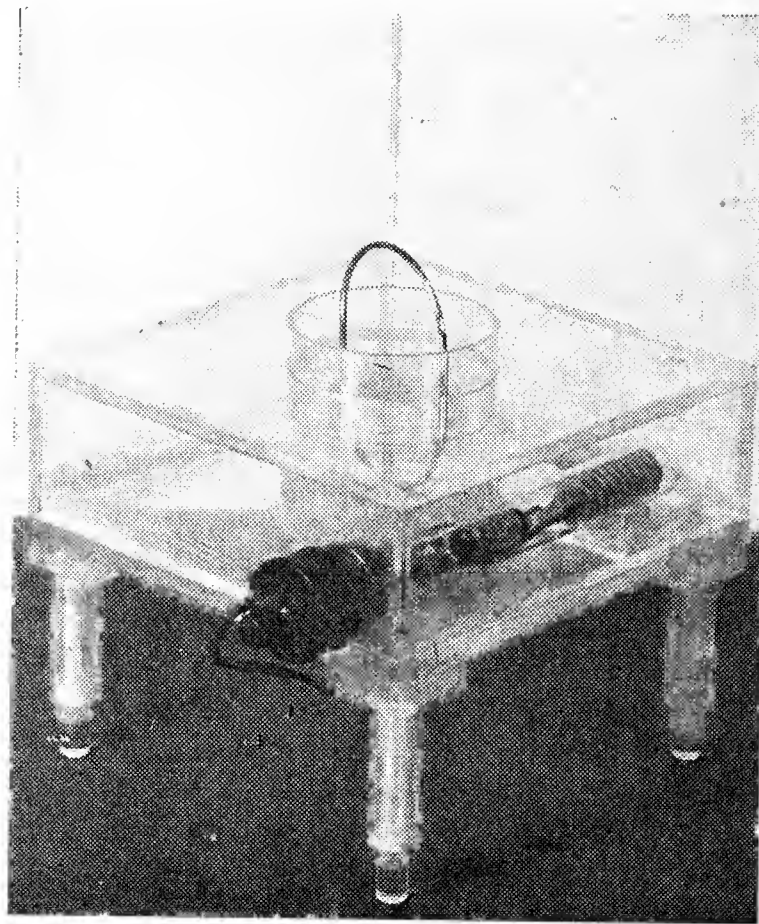


Fig. 1. Single unit plexiglas water bath with flea/tick feeding cup.

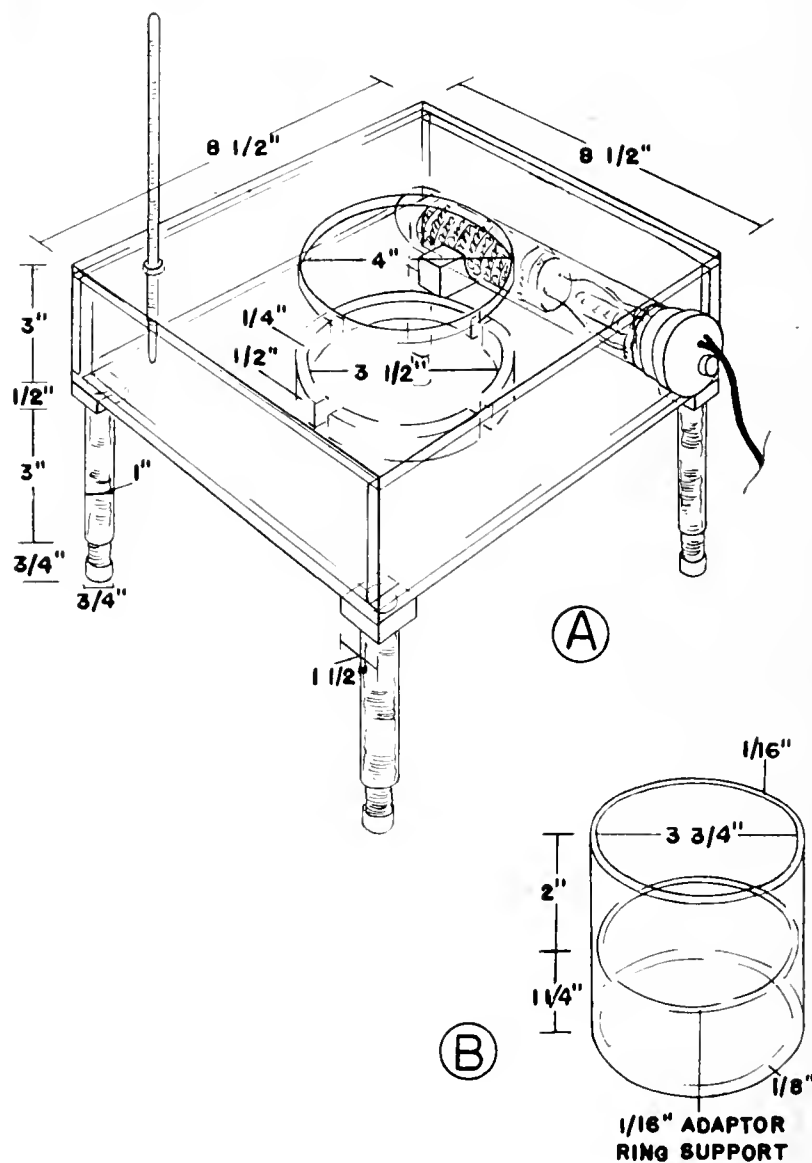


Fig. 2. A. Single unit plexiglas water bath. Walls 1/4-inch thick plexiglas and joints sealed with plastic sealer (trichlorethylene). Each leg composed of two threaded pieces. A 25-watt aquarium immersion heater heats water. B. Plexiglas blood bath for use with single or four unit water bath and any of three feeding cups and adaptor rings. To form 1/8-inch adaptor ring support, 1/16th inch of the inside surface of the top two inches of the blood bath is trimmed away.

soldered over the top opening of the cylinder. Additional rigidity was obtained by soldering a metal ring to the inside bottom edge of the cylinder. For placement and removal of the mosquitoes, a 1/2-inch hole was cut in the center of the lid and a #1 solid rubber stopper was used to close this opening. To hold the cups upright on the blood a stainless steel tube was soldered across the inside of each wire cylinder about an inch below the top. When



in use, a stainless steel rod attached to a ring stand was put through the tubes in each row of cups. These cups were used with the enameled ware pan and open water bath.

The original mosquito membrane cup was revised somewhat when the new flea- and tick-feeding apparatus was built. The wire cylinder and ointment can lid were retained, but the metal cross bar was omitted and a membrane covered plexiglas cup was fitted in the bottom opening and secured with a piece of plastic tape. At times blood was stirred by means of a magnetic stirrer with one teflon-coated magnet in the water bath and one in the blood bath.

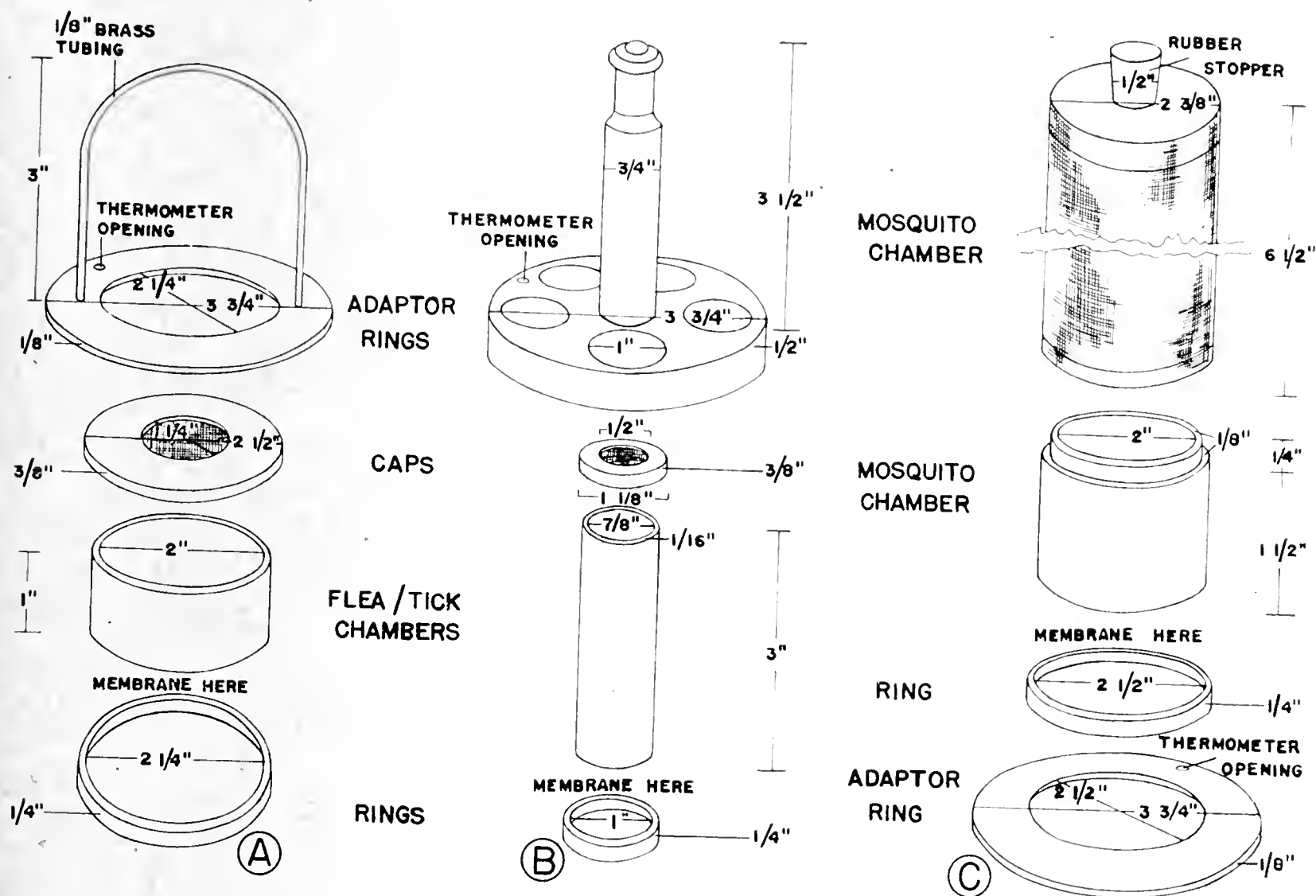


Fig. 3. Plexiglas membrane feeding cups for use with single and four unit plexiglas water baths; all plexiglas 1/8-inch thick unless otherwise specified; Saran, 50-mesh screen cloth secured to caps with plastic sealer. A. Large flea/tick membrane cup apparatus. Brass handles on adaptor ring fastened with brass screws from underneath. B. Five-unit membrane cup apparatus; plexiglas handle fastened to adaptor ring with plastic sealer. C. Mosquito membrane cup apparatus; screen chamber of #20 mesh copper screen cloth.

### MEMBRANES

Rabbit, hamster, guinea pig, and rat skin membranes were obtained from juvenile animals. The animals were killed with chloroform or nembutal, the latter being preferred, and the fur was clipped with a pair of electric animal clippers until only a very fine stubble was left.

To save as much of the tender abdominal skin as possible, the hides of the guinea pigs, hamsters and rats were removed by making longitudinal incisions down the backs of the animals and then carefully cutting the skin away from the flesh. Rabbit skin could easily be removed whole from the animal if the skin around the neck and four legs was cut first. The two front legs then could be tied together with string and the animal hung on a wooden peg or nail making it a simple matter to peel off the skin from the neck down. Next, the skins were stretched out and pinned fur side down on a wooden board and the heavy subcutaneous tissue removed. The finer tissue material was most readily removed if the skins were placed in a deep freeze for several hours, partially thawed, and then scraped with a blunt instrument, such as a Bard-Parker knife handle, until they were translucent. The skins need not be washed in alcohol or running water during or after preparation. Finished skins can be used immediately or left pinned on the wooden boards and stored in the deep freeze for many months. The best rabbit membranes are prepared from the skins of juvenile, male, New Zealand white rabbits. Male animals are preferred since the mammillae of the females often leave holes in the skins after they are prepared.

Very small holes or tears can be repaired successfully with collodion or clear nail polish, which are nontoxic to the arthropods and do not hamper feeding.

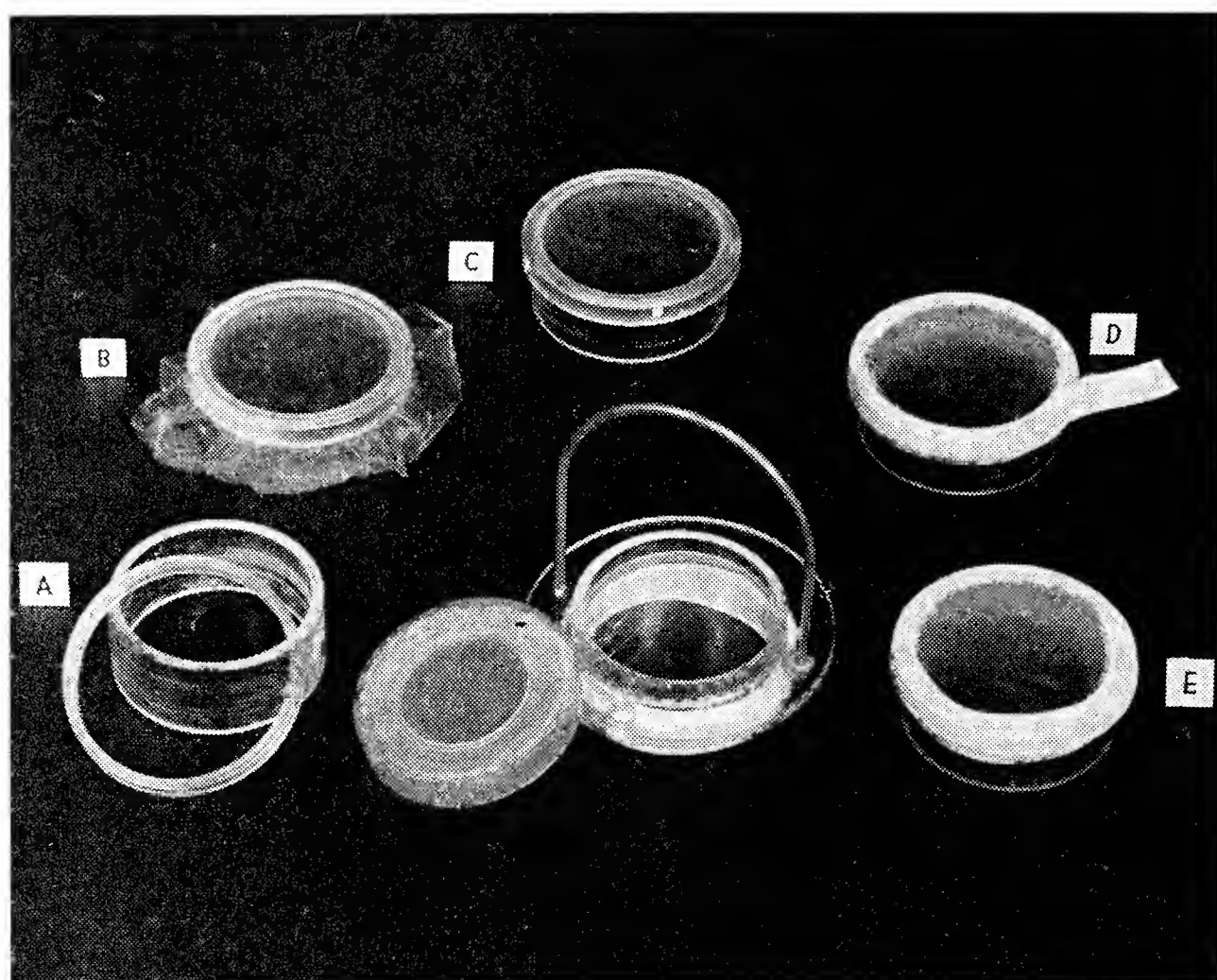


Fig. 4. Assembly of plexiglas membrane feeding cups. A. Ring and cup; B. Ring placed over membrane on cup; C. Membrane cup after membrane trimmed; D. Placement of plastic tape over ring and cup edges; E. Cup with membrane attached ready for use. Center, screened lid of membrane cup and adaptor ring on cup.

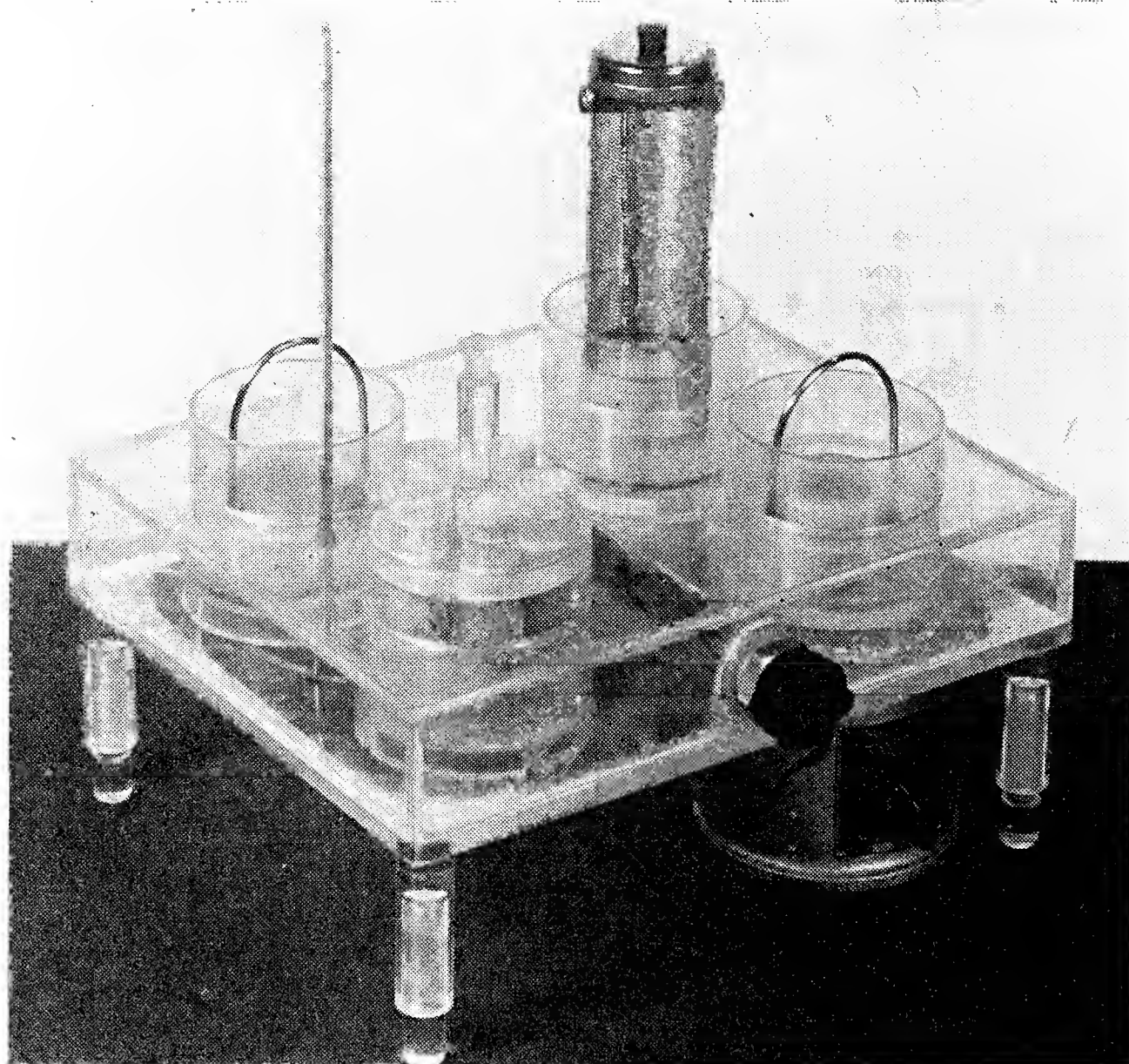


Fig. 5. Four unit plexiglas feeding apparatus.

“Naturalamb”<sup>3</sup> and “Four XXXX”<sup>4</sup> prophylactic skins are prepared commercially from the caecums of lambs. The “Nearkid”<sup>4</sup> and “Zephyr”<sup>4</sup> membranes are prepared commercially from bovine intestines. Beef bladder<sup>5</sup>, beef weasand<sup>5</sup> and unrefined goldbeater skins<sup>6</sup> are prepared, of course, commercially from beeves.

The Baudruche membranes<sup>7</sup> are animal derived and are prepared commercially from bovine intestines. In addition to the Baudruche Transparent membrane, which the writer believes to be the "Baudruche" most generally referred to by other investigators, a number of other Baudruche membranes are manufactured. The thinnest of the Baudruche group is the "Transparent" which is approximately 0.0007 of an inch thick. These skins may be obtained in sheets approximately 9 by 28 to 36 inches. The Transparent membranes sell for \$10.00 per dozen sheets and other types are somewhat higher.

"Silverlight"<sup>8</sup> is the Julius Schmid company trade name for the goldbeater skin obtained from the outermost layer of the caecum of an ox. Silverlight is translucent and extremely thin, being approximately 0.0008 of an inch thick. This membrane may be purchased in sheets 8 by 30 inches at \$5.00 per dozen sheets. The Saran, dialyzing membrane, cellophane and other synthetic products were obtained from various commercial sources. As long as membranes are undamaged, they can be used repeatedly.

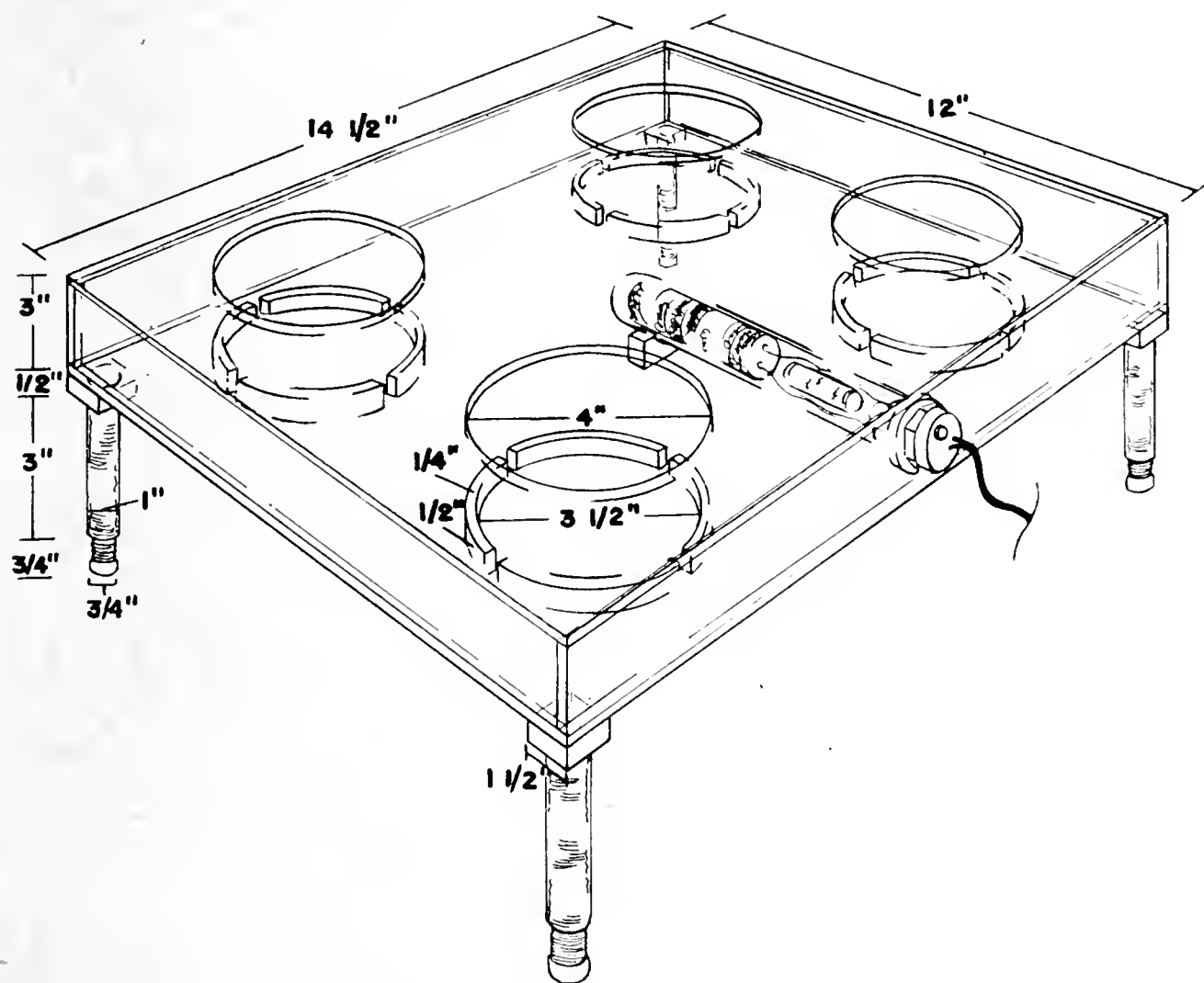


Fig. 6. Four unit plexiglas water bath. All walls of 1/4-inch thick plexiglas and all joints sealed with plastic sealer. Each leg of two threaded pieces. Water bath heated with 25-watt aquarium immersion heater.

## BLOODS AND ANTICOAGULANTS

During the present studies, beef, rat, rabbit, guinea pig, and human bloods were screened for feeding haematophagous arthropods through membranes.

Beef blood was obtained fresh from the abattoir. The jugular vein of a freshly killed steer or cow was pierced with a sterile stainless steel stylet to which a sterile rubber tube was attached. The blood flowed from the vein through the stylet and rubber tube into a sterile 2000 c.c. Erlenmeyer flask containing an anticoagulant. Heparin, sodium oxalate, and sodium citrate were used as anticoagulants. With 100 c.c. of water and 1000 c.c. of

<sup>3</sup>Youngs Rubber Corporation, New York, N.Y.

<sup>4</sup>Julius Schmid, Inc., New York, N.Y.

<sup>5</sup>George A. Hormel and Company, Austin, Minn.

<sup>6</sup>Armour Company, Chicago, Ill.

<sup>7</sup>Long and Long Company, Belleville, N.J.

<sup>8</sup>Julius Schmid, Inc., New York, N.Y.



blood, 0.1 gram of heparin, 1.33 grams of sodium oxalate or 3.33 grams of sodium citrate were used. For the early experimental work the blood was refrigerated for varying numbers of days at 4°C., but refrigerating the blood for more than 1–2 days hindered feeding by some of the arthropods, particularly fleas. Later, therefore, all stored blood was frozen. When thawed and warmed after being frozen as long as 200 days it was as readily ingested by the arthropods as fresh blood. For storage, fresh, heparinized beef blood was filtered through glass wool prior to being poured into pint ice cream cartons, dated and placed in the deep freeze held at –18°C.

Rabbit, guinea pig, and rat bloods were obtained from the animals by cardiac puncture. Approximately 10–13 c.c. of blood can be obtained from a full grown rat, 40–50 c.c. from a full grown guinea pig, and 75–85 c.c. from an adult rabbit. When thawed and warmed, after having been heparinized and frozen for several months, these bloods gave as good feeding results as fresh blood. Outdated human blood obtained from various hospitals and blood banks was used with excellent results.

All frozen bloods were thawed, filtered through glass wool, and pre-heated for the feeding experiments. After pre-heating, the blood was poured in the blood chamber and left until it had remained at a constant temperature for 30 minutes; feeding then started. The feeding apparatus was set up several hours in advance of feeding to allow time for stabilization of the water bath temperature.

### ARTHROPODS

All arthropods used in the experimental work were reared in the laboratory by standard rearing methods.

Newly emerged fleas (*Xenopsylla cheopis*) were sorted and only the most active ones kept. These fleas were stored for several days prior to feeding in a jar which contained a 1/2 to 3/4-inch layer of sand. Just before transfer to the membrane feeding cups the fleas were re-sorted and only the most active ones used. These fleas were anesthetized with CO<sub>2</sub> and transferred from the collecting jar to the membrane cups while the blood was being heated so as to allow sufficient time for revival. The membrane cups were slightly submerged in the blood for feeding. The arthropods were allowed to feed undisturbed.

Mosquitoes (*Aedes aegypti*) were collected from the holding cages with an aspirator just prior to feeding and while the blood was heating. They were anesthetized with CO<sub>2</sub> in the aspirator collecting jar, placed in the membrane cups and the cups placed on the blood as for the fleas. Mosquitoes were given no sugar for 48 hours and no water for 24 hours prior to feeding.

Ticks (*Ornithodoros moubata*), just prior to feeding and while the blood was heating, were anesthetized with CO<sub>2</sub> in the jars in which they had been stored and then transferred to the membrane cups. The cups were placed on the blood as for flea feeding.

At the conclusion of each feeding experiment, the membrane cups were removed from the blood, the excess blood sponged from the bottoms of the cups and the arthropods anesthetized with CO<sub>2</sub>. Fleas were sorted on revival and the most active were collected in a vial, again slightly anesthetized with CO<sub>2</sub> and examined.

## RESULTS

### BLOOD TEMPERATURE

It has been reported that various haematophagous arthropods have been successfully fed on blood warmed to temperatures of 36–45°C. (Bishop and Gilchrist, 1948; De Meillon, *et al.*, 1948; Fuller, *et al.*, 1949; Trembley, 1952; Kartman, 1954; Haddon, 1956). Early in the present studies a blood temperature of 36°C. was used. Subsequent experiments showed that fleas and mosquitoes fed best at 34°C. (Table II). No blood temperature comparison tests were done with ticks, but these arthropods fed well on blood held at 34°C.

### AMBIENT TEMPERATURES

Haddon (1956) found that the human body louse (*Pediculus humanus corporis*) fed best when ambient temperatures were 26–30°C. The author found that fleas, ticks, and mosquitoes fed very poorly when ambient temperatures rose above 30°C. However, when the feeding apparatus was placed in an atmosphere of 19–30°C. results were good. When



TABLE II. Feeding Results With Beef Blood Held at Varying Temperatures.\*

Membrane Used	Blood Temperature (°C.)	Number Fed/Exposed	% Fed
	<i>X. cheopis</i> **		
Rabbit	32	433/511	84
Rabbit	34	464/482	96
Rabbit	36	394/512	80
Rabbit	38	381/501	76
Rabbit	40	183/430	41
	<i>A. aegypti</i> ‡		
Silverlight	32	167/240	70
Silverlight	34	210/235	90
Silverlight	36	181/241	75

\*Arthropods were allowed to feed for 60 minutes in the feeding apparatus.

\*\*Fleas starved from 144 to 192 hours before feeding.

‡Mosquitoes were 4 to 6 days old and given no sugar solution for 48 hours and no water for 24 hours before feeding.

the ambient temperature rose above 30°C. containers of ice were placed near the feeding apparatus to lower the temperatures.

#### BLOOD ANTICOAGULANTS

The effects of various blood anticoagulants on feeding, and the possibility of the anticoagulants being toxic to the arthropods were investigated. Tests with heparin, sodium oxalate, and sodium citrate (Table III) showed that all other factors in the experiments being equal, any one of the three anticoagulants could be used without apparent diminution of feeding by the arthropods, and the arthropods so fed remained alive throughout the feeding. In studies on survival, fleas fed on heparinized blood remained alive for 3 to 8 weeks with and without subsequent blood meals. In a few experiments mosquitoes were fed on defibrinated beef blood, but the feeding results (70 per cent fed) were not as good as in experiments with whole blood containing heparin, sodium oxalate, or sodium citrate.

TABLE III. Feeding Results With Various Anticoagulants in Beef Blood.\*

Membrane Used	Blood Temperature (°C.)	Anti-Coagulant	Number Fed/Exposed	% Fed
		<i>X. cheopis</i> **		
Rabbit	36	Heparin	359/406	88
Rabbit	36	Citrate	401/450	89
Rabbit	36	Oxalate	364/427	85
		<i>Aedes aegypti</i> ‡		
Silverlight	34	Heparin	111/130	85
Silverlight	34	Citrate	159/199	80
Silverlight	34	Oxalate	123/147	84

\*Arthropods were allowed to feed for 60 minutes in the feeding apparatus.

\*\*Fleas starved from 144 to 192 hours before feeding.

‡Mosquitoes were 4 to 6 days old and given no sugar solution for 48 hours and no water for 24 hours before feeding; ambient temperature 19 to 30°C.

#### FEEDING TIME

From 45 to 60 minutes was determined to be the most practical and generally optimum period of time to allow the arthropods opportunity to ingest blood through a membrane. Table IV gives the results of feeding time experiments with fleas.

TABLE IV. Feeding Results With *Xenopsylla cheopis* After Varying Time Intervals Utilizing Rabbit Skin Membranes.\*

Feeding Time Allowed (Minutes)	Number Fed/Exposed	% Fed
10	313/514	61
15	417/543	77
20	311/529	59
30	820/1162	71
45	151/163	93
60	454/489	93
90	1225/1276	96

\*Fleas starved from 144 to 192 hours before feeding; blood temperature: 34° C.; ambient temperature: 19 to 30° C.

#### MEMBRANE THICKNESS

Fuller, *et al.* (1949) noted that membrane thickness may be a determining factor in the feeding of lice, and Kartman (1954) found that thin rat skin membranes gave the best feeding results for fleas. Contrariwise, Wheeler, *et al.* (1956) reported that rat skins merely removed from the animals and used without further preparation yielded as good feeding results with fleas as the more meticulously prepared membranes used by other workers.

Membrane thickness experiments were done by the author with rabbit skins and fleas. The original flea-feeding experiments with rabbit skins gave very erratic results and it was soon found that this was due to the variation in the thickness of the membrane. Rabbit skin membranes were thereafter measured with a micrometer for average thickness and final results showed that membranes more than 0.013 inches thick gave unsatisfactory results (Table V).

TABLE V. Feeding Results With *Xenopsylla cheopis* Utilizing Rabbit Skin Membranes of Varying Thickness.\*

Membrane Thickness (Inches)	Number Fed/Exposed	% Fed
.008	1883/2280	83
.009	332/342	97
.010	529/708	75
.011	2201/2834	78
.012	648/794	82
.013	1948/2299	85
.015	116/211	55
.017	85/224	38
.019	34/235	15
.021	34/239	14
.023	4/218	2
.025	9/240	4
.028	5/231	2
.030	0/192	0

\*Fleas starved 144 to 192 hours before feeding; blood temperature: 34° C.; ambient temperature: 19 to 30° C.

#### AGE OF ARTHROPODS

Kartman (1954) got 84.3 per cent of 237 fleas to become engorged when they were fed after having been starved for 72 to 96 hours following emergence. This phase of the present experimental work was done only with fleas and it was found that they fed best when a starvation period of 144 to 288 hours followed emergence and preceded feeding (Table VI).

TABLE VI. Feeding Results With *Xenopsylla cheopis* of Varying Ages Utilizing Rabbit Skin Membranes.\*

Age of Starved Fleas (Hours)	Number Fed/Exposed	% Fed
24	94/481	20
72	200/495	40
96	399/500	78
120	377/499	76
144	406/485	84
168	444/499	89
192	468/489	96
216	455/510	89
240	380/411	93
288	335/470	93

\*Blood temperature: 34° C.; ambient temperature: 19 to 30° C.; feeding time 60 minutes.

## STIRRING OF BLOOD

During the early experiments, the blood always formed a thickish layer on the under-side of the membrane. Since this caking was thought to be due to the membrane standing so long in the blood, it seemed desirable to stir the blood during feeding. Experiments were done in which some fleas were allowed to feed on blood being stirred and some on stationary blood. The blood was stirred at a slow rate of speed, between the 4 and 5 setting of the magnetic stirrer. Concurrently with the blood experiments a comparison study was run on feeding results with membranes attached tautly and loosely.

The stirred versus stationary blood experiments and subsequent work revealed no drastic differences in results. The experiments on tautly and loosely attached membranes gave comparable results with all the rodent membranes. However, tautly attached Silver-

TABLE VII. Feeding Results With *Xenopsylla cheopis* on Various Membranes.

Membranes	Number Fed/Exposed	% Fed	Feeding exps.
Hamster skin	1079/1112	97	9
Rat skin	2369/2486	95	15
Baudruche transparent	1279/1361	94	8
Silverlight	17572/20783	85	81
Rabbit skin	35455/43219	82	182
B-Yellow transparent*	129/241	62	1
B-Zephyr single*	312/511	61	4
Beef Weasand (Unsalted)	160/331	48	3
Guinea pig skin	119/258	46	3
B-Zephyr Double*	56/183	31	3
B-Single Dark Yellow*	112/441	25	4
Goldbeater (salted)	45/218	21	1
Goldbeater (unsalted)	69/358	19	4
Beef bladder	15/141	11	1
Chick skin	20/194	10	2
Nearkid	11/211	5	1
Patrician (prophylactic skin)	4/142	3	1
FourXXXX (prophylactic skin)	6/173	3	1
B-Double Dark Yellow*	0/200	0	2
B-Light Double Yellow*	0/200	0	2
Zephyr	0/100	0	1
Saran	0/200	0	2
Transparent plastic	0/100	0	1
Naturalamb (prophylactic skin)	0/112	0	1
Beef Weasand (salted)	0/102	0	1

\*Baudruche Membranes.

light membranes gave consistently lower feeding results, regardless of whether the blood was stirred or stationary. Additional experiments have shown that the extreme thinness of Silverlight makes it necessary to exercise extra care in attaching it to the cups and in preparing the blood to be used with it. It must be attached loosely, but not so loosely as to allow an air pocket to form between the blood and the membrane. Additionally, while it is particularly advisable to strain blood through glass wool just prior to heating for feeding through Silverlight, this is a recommended procedure for any blood to be used with any membrane. Results showed that a slight caking of blood on the membrane does not hamper feeding.

#### FEEDING RESULTS WITH VARIOUS MEMBRANES

Of the 25 membranes screened for flea feeding, hamster, rabbit, rat, Baudruche Transparent, and Silverlight membranes gave the highest feeding results. Silverlight gave the best results for mosquitoes in experiments with 21 membranes, and rabbit skin proved superior to 9 other membranes for ticks. Results of these experiments will be found in Tables VII, VIII and IX. Table X gives details of experiments with fleas and the five best flea membranes.

TABLE VIII. Feeding Results With *Aedes aegypti* on Various Membranes.\*\*

Membranes	Number Fed/Exposed	% Fed	Feeding exps.
Silverlight	2881/3833	75	63
B-Black Transparent*	85/148	57	3
B-White Opaque*	62/117	53	3
B-Yellow Transparent*	80/154	52	3
B-Single Dark Yellow*	75/155	48	3
B-Zephyr Double*	59/169	35	3
B-Transparent*	69/196	35	1
B-Light Double Yellow*	52/161	32	3
B-Zephyr Single*	42/148	30	3
Goldbeater (salted)	53/189	28	3
B-Double Dark Yellow*	35/147	24	3
Rabbit skin	86/699	12	14
Four XXXX (prophylactic skin)	7/51	14	1
Chick skin	5/50	10	1
Beef bladder	5/53	9	1
Dialyzing Membrane	3/49	6	1
Fish Swim Bladder	3/73	4	1
Naturalamb (prophylactic skin)	2/55	4	1
Beef Weasand (unsalted)	1/56	2	1
Beef Weasand (salted)	0/46	0	1
Patrician (prophylactic skin)	0/85	0	1

\*Baudruche membranes.

\*\*Mosquitoes were 4 to 6 days of age and given no sugar solution for 48 hours and no water for 24 hours before feeding; feeding time 60 minutes; blood temperature: 34° C. except in experiments listed in Table II.

#### REMARKS

The present investigations showed that ambient temperature, membrane derivation and thickness, blood temperature and storage, and age and degree of starvation of the arthropod were the most critical factors in membrane feeding of arthropods. There are, as has been mentioned, many other factors that enhance or detract from feeding results but these are of secondary importance when the above listed factors are optimum. The importance of ambient temperature in membrane feeding makes the use of enclosed water and blood baths essential as it would be almost impossible to maintain a sufficiently low ambient temperature while retaining a sufficiently high blood temperature in an open bath. If ice is used to control the ambient temperature it is safest to use ordinary ice rather than dry ice. When dry ice is used in a confined space, such as a biological hood, enough carbon dioxide accumulates to anesthetize or sometimes kill the arthropods.

No recordings were taken on ambient humidity, but it seems certain that this is not a critical factor.



TABLE IX. Feeding Results With *Ornithodoros moubata* on Various Membranes.\*\*

Membranes	Number Fed/Exposed	% Fed	Feeding exps.
Rabbit skin	68/70	97	2
Goldbeater (salted)	23/46	50	1
Naturalamb (prophylactic skin)	64/149	43	4
B-Yellow Transparent*	16/40	40	1
B-Single Dark Yellow*	55/144	38	4
B-Zephyr Double*	13/37	35	1
B-Black Transparent*	51/148	34	4
Silverlight	24/113	30	3
Four XXXX (prophylactic skin)	6/26	23	1
Beef Weasand (unsalted)	2/27	7	1
B-Light Double Yellow*	0/40	0	1

\*Baudruche membranes.

\*\*Ticks were seven-day-old unfed nymphs; feeding time 60 minutes; blood temperature 34° C.; beef blood used for feeding ticks.

TABLE X. Results of Feeding *Xenopsylla cheopis* on Five Best Membranes.\*

Membranes	Age of Fleas (Hours)	Blood	Blood Temp. (°C.)	Ambient Temp. (°C.)	Number Fed/Exposed	% Fed	Feeding exps.
Silverlight	144-240	Beef	32-42	19-30	14467/17070	85	69
Silverlight	144-288	Rat	33-35	19-30	1937/2463	78	10
Silverlight	192	Human	34	26	234/250	94	1
Silverlight	168	Guinea pig	34	26	934/1000	93	1
TOTAL					17572/20783	85	81
Baudruche Transparent	144-216	Beef	34-36	19-30	875/937	93	5
Baudruche Transparent	144-192	Rat	34-35	26-30	292/304	96	2
Baudruche Transparent	96	Human	34	26	112/120	93	1
TOTAL					1279/ 1361	94	8
Rat skin	144-192	Rat	34-36	19-30	650/713	91	4
Rat skin	144-216	Beef	34-36	19-30	1719/1773	97	11
TOTAL					2369/2486	95	15
Hamster skin	144-216	Beef	34-35	19-30	977/1010	97	8
Hamster skin	144	Rat	34	26	102/102	100	1
TOTAL					1079/1112	97	9
Rabbit skin	24-456	Beef	32-40	19-30	35360/43119	82	181
Rabbit skin	144	Rat	34	27	95/100	95	1
TOTAL					35455/43219	82	182

\*All fleas were fed for 60 minutes except 4187 that were fed on beef blood through rabbit skin membranes in the feeding time experiments (see Table IV).

Arthropods should not be placed in the membrane cups until just before use or, if they must be prepared ahead of time, the cups should be kept in a temperature and humidity identical to those at which the arthropods were stored. On a few occasions when fleas were placed in membrane cups in the morning, those given an opportunity to feed in the morning, shortly after being placed in the cups, fed better than those fed in the afternoon after having been left out in the room for several hours.

Though no experiments were done to compare feeding by female and male fleas, on a few occasions while fed fleas were being counted it was noted that both sexes fed equally

well. No determinations were made as to the actual quantity of blood ingested, but during counting it was noted that those fleas that did feed fed to completion.

With the exception of rat, rabbit, and chick skins, Baudruche Transparent, transparent plastic, and Saran, the membranes screened in this investigation were new for *in vitro* feeding research. While only a few of the 23 new membranes proved to be highly satisfactory, many of the others were insufficiently tested and still should be thoroughly screened. From the feeding results obtained with rat, rabbit, and hamster skin membranes it appears that any rodent or similar skins could be used with success provided they are very thin. However, the time necessary for preparing the skins and the cost of the animals makes the use of commercially prepared membranes advantageous when feeding results are comparable.

Though a number of the synthetic membranes are as thin as the Silverlight and Baudruche Transparent membranes, their use is not feasible since their tough composition prevents the arthropods from penetrating them. The animal-derived membranes become soft and pliable and easily penetrable by arthropods when placed on liquids.

### ACKNOWLEDGEMENTS

The writer is indebted to the following personnel at Fort Detrick: Mrs. Anita Cooley Wilson, Mr. William Hilsenhoff, Mr. Robert Wagner, Mr. Robert Morgan, Dr. Mitchell Byrd, Mr. Earl Oden, Mr. Roland Staley, Mr. Joseph Carroll, Mr. Charles Marman, Mr. R. T. Miller, Mr. Robert Castle, Mr. George Evans and Mr. George Hess for their technical assistance. Acknowledgement also is due Mr. Julius Schmid, Jr., of the Julius Schmid Company, for sending samples of Silverlight and other membranes, Mr. Joseph Long of Long and Long Company for the Baudruche membranes, and to numerous others who aided in the investigative work.

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## DISCUSSION

R. M. GORDON. (1) Can the apparatus described be used for feeding arthropods under sterile conditions? (2) Silverlight membranes; where are these obtainable?

I. B. TARSHIS. (1) I have never worried about sterilization since the apparatus cannot be sterilized because it is made of plexiglas. (2) From Julius Schmid, Inc., 423-439 West 55th St., New York 14, N.Y.



# Periodicity of Biting Behaviour of Some African Mosquitoes

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## ABSTRACT

The biting cycle of *Aedes aegypti* was defined by baited catches made continuously throughout the 24 hours on native boys in, and on the verandah of, a hut in Newala District, Tanganyika. There were four main peaks of activity inside the hut, at 03, 08, 14 and 21 hours. The biting curve on the hut verandah showed some differences, particularly the occurrence of a wave about sunset. Teesdale (1955) working in 4 environments at Mombasa, Kenya, found essentially only two main periods of activity, one of low intensity and long duration in the morning, and another, more abrupt and generally reaching higher intensity levels, about sunset.

Other examples of differences in biting cycle for the same species of mosquito in different environments are cited. *Aedes simpsoni* showed much less morning biting activity in forest at Taveta, Kenya, than in plantation in Bwamba, Uganda. *Aedes pembaensis* showed two peaks of biting activity, near dusk and dawn, at its breeding places, only one, the dusk peak, away from them. Deane, Causey and Deane (1948), working with South American anophelines, appear to have been the first to record such differences in biting activity with environment; for example, they found the activity of *Anopheles albitarsis* in the open air to occur in two waves, with peaks about dusk and dawn, while in houses there was a single wave of activity about the middle of the night. This type of difference has recently appeared for *Taeniorhynchus fuscopennatus* inside and outside forest in Uganda.

The mechanisms which might determine these differences are discussed in relation to these and other examples: (a) The differences are associated with differences in the genetic composition of the species. (b) The differences are the result of differences in microclimatic factors between environments. (c) The possible effects of differences in the age composition of populations (as age has been shown to be associated with differences in biting habits in *Simulium*), of the periodicity of other activities such as oviposition, and of the relation of the catch site to the site of other activities are pointed out.

The continuous baited catch made with native mosquito catchers, as originally devised by Kerr (1933) and elaborated and standardized by Kumm and Novis (1938) and, mainly, by Haddow (1954) has come into increasing use as a tool for the study of the behaviour of insects of importance in the epidemiology of tropical parasitic infections. It is the purpose of the present paper to discuss one aspect of the now considerable body of information which the method has yielded, viz., the finding of biting activity curves of different form for the same species in different environments.

The biting activity of *Aedes* (*Stegomyia*) *aegypti* (L.) was studied in the interior and on the verandah of a hut in Newala District, Tanganyika, by means of the continuous baited catch (Lumsden; in press). The catch lasted for only two days but the species was extremely abundant and the curves (Fig. 1) are based on 1973 and 621 specimens, respectively. Inside the hut there were four main waves of activity and these waves appeared to be consistent, each one occurring twice in the course of the two days of catching. Two of the waves occurred by night and two by day and, beginning from midnight, they may be referred to as the first to fourth main activity waves, with peaks at about 03–04, 08–09, 14–15 and 21–22 hours. Besides the four main activity waves mentioned there are also signs of subsidiary activity waves, about dawn and about midnight.

The biting activity curve for the hut verandah shows general similarity to that for the hut interior, but also some differences. Activity appeared to occur in six waves, again largely consistent in their occurrence in the two days of the catch. Some of these corresponded with the waves of activity inside the hut, some did not:—

- (a) The first wave, in the hour 02–03, is close to the first main wave in the hut interior.
- (b) The second wave, at about dawn, is close to the subsidiary wave which occurs in the hut interior at about this time.
- (c, d) The third and fourth waves correspond fairly closely to the second and third main waves in the hut interior.

- (e) The fifth wave, at about sunset, is not represented in the hut interior.
- (f) The sixth wave, at about midnight, corresponds with the subsidiary wave which occurs at about this time in the hut interior.

Teesdale (1955) has studied the activity of *A. aegypti* in 4 different environments at Mombasa, Kenya, inside and outside a house, under a tree, and in bush. He found essentially the same rhythm in all these four environments, viz., two main periods of activity, one tending to be of lower intensity and longer duration in the morning hours and another, more abrupt and generally reaching higher activity levels, at about sunset (Fig. 2). Thus Teesdale's results seem to define two of the waves found in our own, the morning one, which occurred both inside and outside the hut, and the sunset one, which occurred outside the hut only. The sunset wave was generally much more conspicuous than at Newala, and in three out of four environments provided the highest biting activity of the whole 24-hour period. Some of the other activity waves found at Newala are faintly indicated in some of Teesdale's series of figures.

Thus, considering all the work together, it does appear that the time of occurrence of the activity waves of *A. aegypti* are fairly well defined and that the several forms of the activity curve are fundamentally similar, their differences being mainly the result of accentuation or suppression of different waves. Some other examples of differences in biting activity cycles for the same species of mosquito in different environments are considered below.

Haddow (1945) showed that the biting activity of *A. (Stegomyia) simpsoni* Theobald in banana plantation in Bwamba, Uganda, occurs in two main waves of about the same size, by day, in the morning and afternoon. The two waves are separated by a period of low activity which falls at 13–14 hours (Fig. 3). However, in forest at Taveta, Kenya, where the species was abundant both at ground level and on the tree platform, its biting activity curve differed in that the two waves were of very different size, that in the afternoon being much more conspicuous than that in the morning (Fig. 3) (Lumsden, 1955).

*Aedes (Skusea) pembaensis* (Theobald) affords another example (Lumsden, 1955). Its larvae occur in crab holes in the *Avicennia* mangrove belt of the Kenya coast. Fig. 4 shows the cycle of biting activity found in that environment and in another only a few hundred yards away. At the breeding places there were two main activity waves, one in the first half of the night with a peak in the hour 19–20 and another in the second half of the night reaching a maximum just after dawn (Fig. 4). Away from the breeding places the evening wave was accentuated and included practically all the activity of the whole 24-hours, the morning wave being absent. Although these waves are based on only 3 and 2 days catching, respectively, the numbers are not inconsiderable and conditions of weather, shelter, etc., were similar in the two environments. Also a parallel relationship occurred in moving catches. The morning catch per man-hour was 10 times the evening one at the breeding places only 0.7 of it away from them.

The examples quoted are not the first to be recorded. These appear to be the curves recorded by Deane and others (1948) for some South American *Anopheles*, which are

Fig. 1. Biting activity curves for *Aedes (Stegomyia) aegypti* (L.) in a hut and on its verandah; Newala District Tanganyika. Each curve is based on 49 hours catching, yielding respectively 1873 and 621 mosquitoes (Lumsden, 1957).

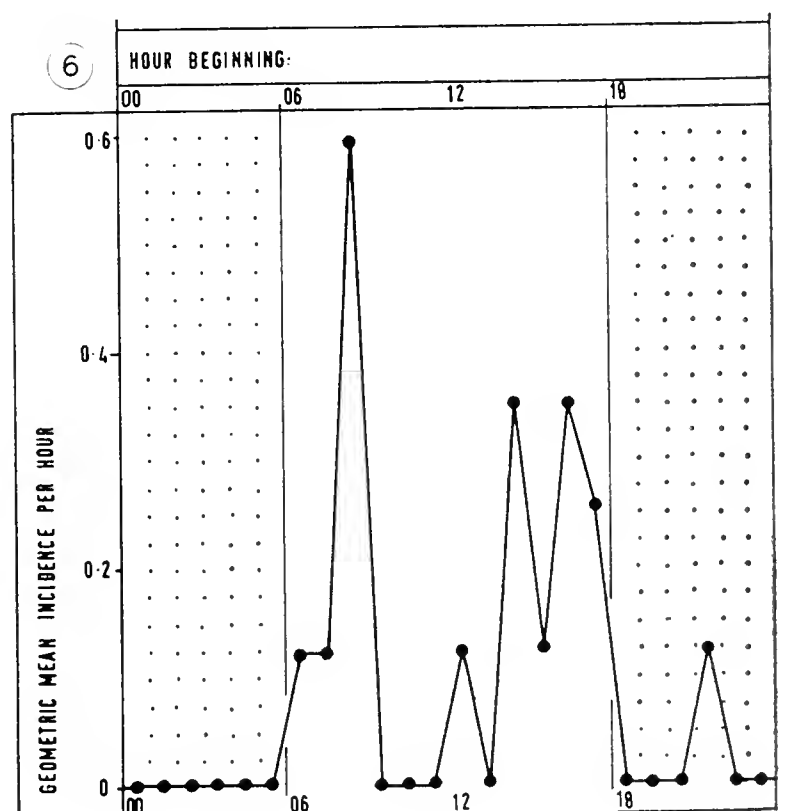
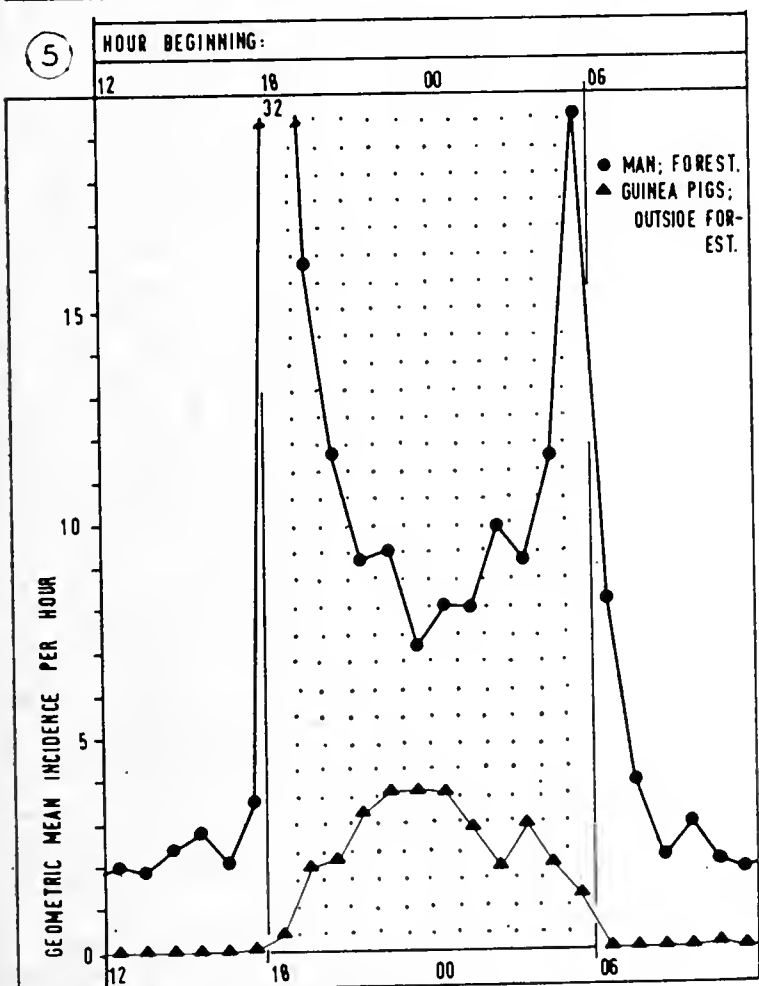
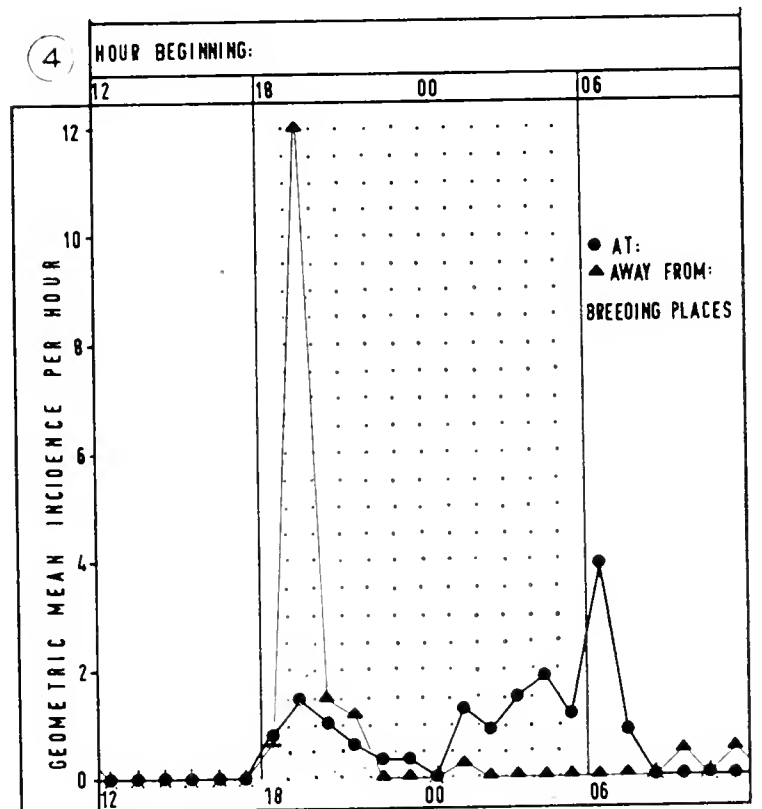
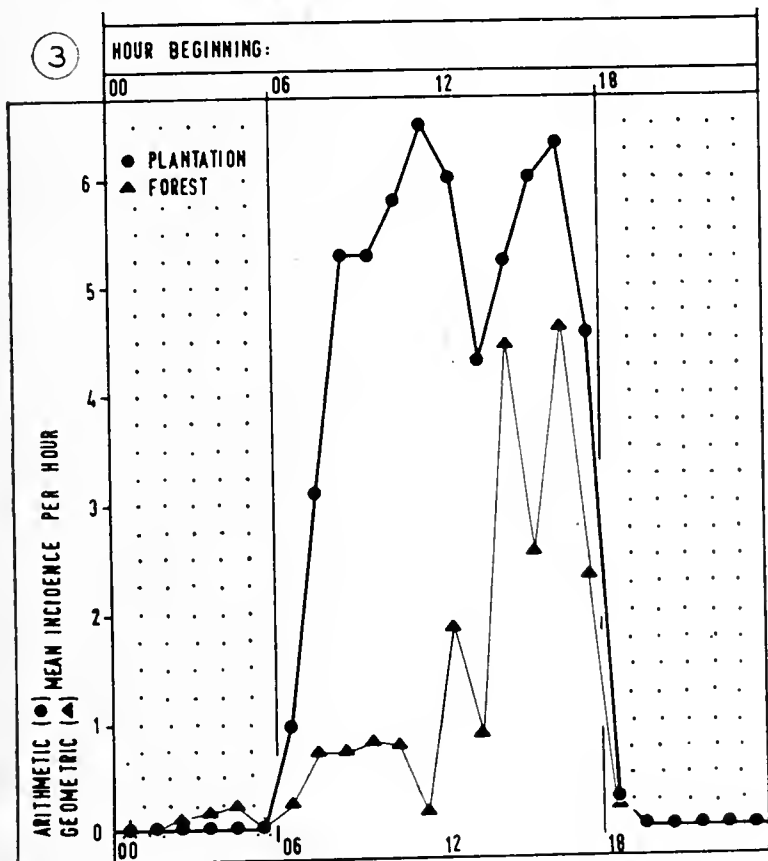
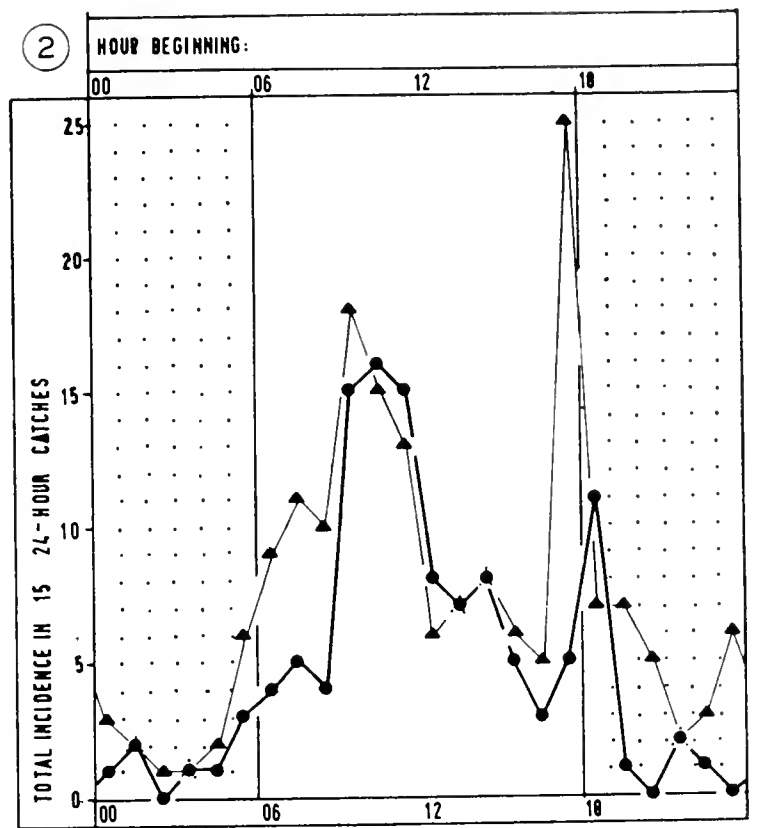
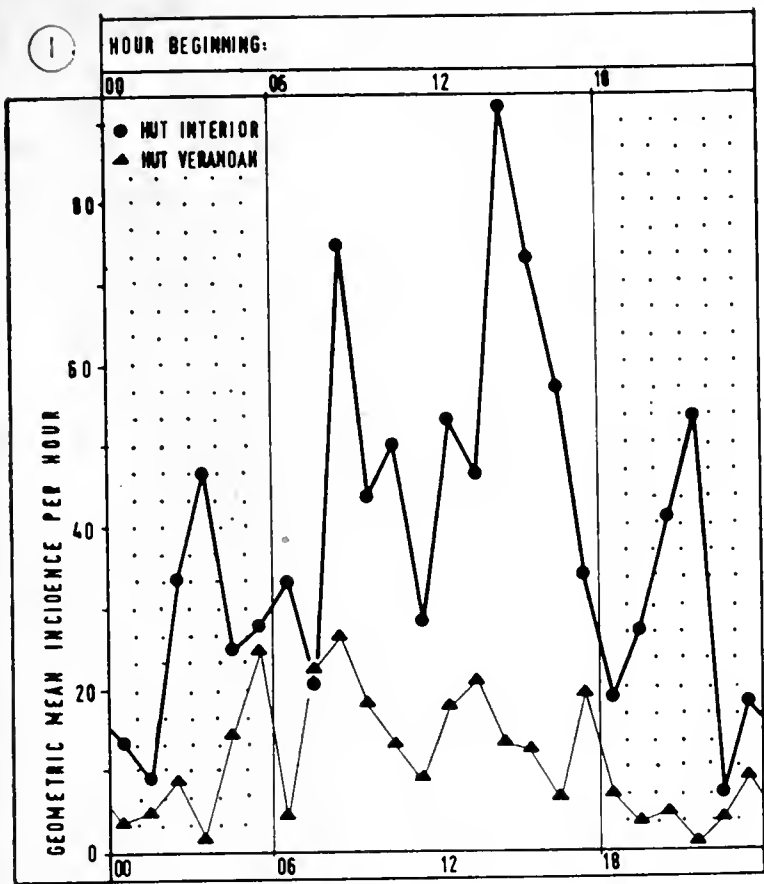
Fig. 2. Biting activity curves for *Aedes (Stegomyia) aegypti* (L.) in a hut and outside it; Mombasa, Kenya. Each curve is based on 15 days catching, 118 mosquitoes being taken in the hut and 179 outside it (Teesdale, 1955).

Fig. 3. Biting activity curves for *Aedes (Stegomyia) simpsoni* (Theobald) in banana plantation, Uganda, and in forest, Kenya. Based, respectively, on 10 days catching (596 mosquitoes) (Haddow, 1945), and 5 days catching (314 mosquitoes) (Lumsden, 1955).

Fig. 4. Biting activity curves for *Aedes (Skusea) pembaensis* (Theobald) at its breeding places in *Avicennia* mangrove forest, and a few hundred yards from its breeding places, in bush; Gede, Kenya. Based respectively on 3 days catching (70 mosquitoes) and 2 days catching (40 mosquitoes) (Lumsden, 1955).

Fig. 5. Biting activity curves for *Taeniorhynchus (Coquillettidia) fuscopennatus* Theobald in forest on human bait, and outside forest on guinea pigs. Based respectively on 60 days catching (11,461 mosquitoes) and 6 days catching (223 mosquitoes) (Haddow, unpublished; Lumsden, unpublished).

Fig. 6. Biting activity curve for *Aedes (Stegomyia) africanus* Theobald for ground and 12 m. levels, combined, in forest; Taveta, Kenya. Based on 3 days catching (19 mosquitoes) (Lumsden, 1955).



markedly different, depending on whether the catches were made inside or outside houses. In the case of *A. aquasalis* Curry, the activity outdoors occurs in two sharp waves, one soon after sunset and the other about midnight. Indoors there is a single slowly rising and falling wave with a peak in the second half of the night. Even more remarkable are the curves given by these workers for *A. albitarsis* Lynch-Arribalzaga, in which the outdoor activity occurs in two waves with peaks about dusk and dawn while indoors there is a single wave with a peak near the middle of the night, when the other curve is near its lowest point.

Curves closely similar to those given by Deane and others for *A. albitarsis* have recently appeared in the case of *Taeniorhynchus* (*Coquillettidia*) *fuscopennatus* Theobald (Fig. 5). In this species the activity curve in forest, at all levels, though most markedly at the upper ones, consists of two main waves with peaks about dusk and dawn, closely similar to the out-of-doors curve for *A. albitarsis* (Haddow; unpublished). A few hundred yards from forest, in the compound of the Virus Research Institute in a sheltered position under trees, an hourly trap catch on guinea pigs gave a result similar to the indoors curve for *A. albitarsis* (Fig. 5).

With regard to the underlying mechanisms determining these different biting activity curves, three main possibilities come to mind to be discussed:—

(a) The differences are associated with differences in the genetic composition of the species. Such an explanation was considered a likely one in the case of *Aedes simpsoni* at Taveta until Haddow found, on scrutinizing the sparse occurrences of the species in forest in Bwamba, Uganda (see Lumsden, 1955) that the same form of curve occurred, indicating that reduction or suppression of morning activity in this species is characteristic of the forest environment. Haddow (1954) records an even more marked difference for *Anopheles implexus* Theobald which in one part of Uganda is largely nocturnal, in another largely diurnal. But even so fundamental a difference as this should only with hesitation be ascribed to a racial difference in behaviour. The biting activity curve for *Aedes africanus* at Taveta, Kenya, (Lumsden, 1955) may be considered (Fig. 6). This curve, though founded on only small numbers as the species was rare, was strikingly different from that established as the normal by Haddow and others (1947), Mattingly (1949), and Lumsden (1952), in which practically all the 24-hours' activity occurs in a single abrupt wave with a peak between 20 and 30 minutes after sunset. At Taveta, both at ground level and at 13 m., activity was mainly diurnal and the composite curve bears some resemblance to that typical of *Aedes simpsoni* with waves of activity in the morning and afternoon. It appeared likely that this difference might be racial, particularly as the *A. africanus* population at Taveta is a relict one, rather widely separated from the main distribution of the species. However, it is now clear that this type of curve is characteristic of *A. africanus* at the lower forest levels (Haddow (1954); Williams, personal communication), and the platform level at Taveta, where there were many giant trees, may not have been high enough to enter the level where the activity cycle was of the more usual type.

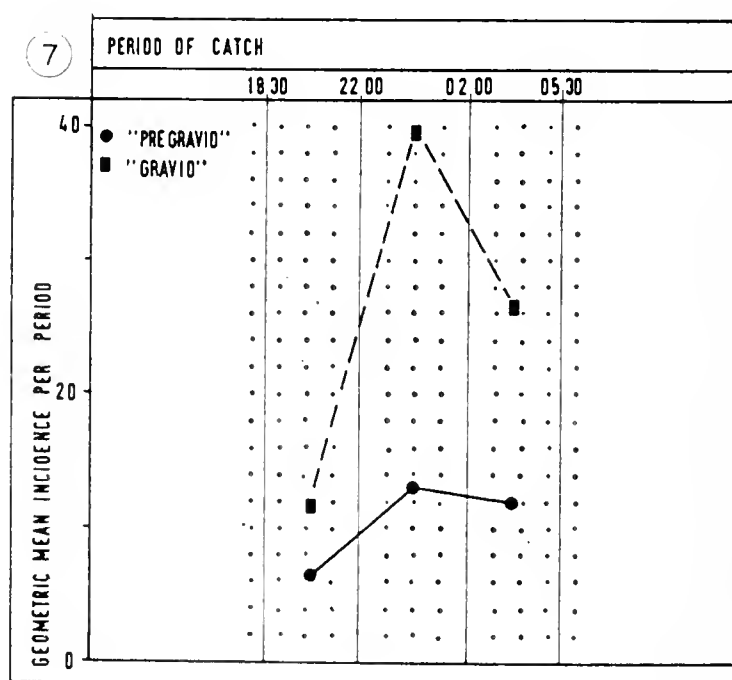


Fig. 7. Biting activity curves for two sections of the population, "pregravid" and "gravid", of *Anopheles gambiae* Giles; Tengeni, Tanganyika. Based on 22–24 catches (5154 mosquitoes) (Gillies, in press).



(b) The differences are the results of differences in microclimatic factors between environments. Some factors, such as wind, are well-known to depress mosquito activity as, for example, *Anopheles gambiae* and *Aedes africanus* in forest in Uganda (Lumsden, 1952). Temperature, humidity, and light have been studied and these factors all seem most likely to be effective at about sunrise and sunset and through the day. It seems possible, for instance, that the virtual absence of the morning wave of activity in *Aedes simpsoni* in forest (Fig. 3) is due to the slower rise of temperature in that environment as compared with the more open banana plantation. However, microclimatic factors do not appear to be sufficient to account for many of the changes, particularly the waves of activity which occur by night when, between the twilight periods, conditions tend to be very uniform, particularly in such environments as in forest and in huts.

(c) It appears possible that other considerations may be of importance. Lumsden (1952) suggested that if different groups of the population, perhaps age groups, tended to bite at particular times, then the form of the curve might be expected to vary with the age composition of the population. Davies (1955) has actually reported a difference in feeding habits with age in *Simulium* but, at least as far as some anopheline species are concerned, it is clear (Senior White, 1953; Gillies, in press) that effects of age in mosquitoes, if they occur, are not clear cut. However, at least one interesting point has emerged. Gillies (in press) separated "pre-gravid" (mostly feeding for the first time) from "gravid" (feeding for the second and subsequent times) *Anopheles gambiae* in huts in Tanganyika. Over an 8-week period, though there was marked fluctuation from week to week, there was little difference in the arithmetic representation of "pregravids" through the night, it being, for 3 approximately 4-hour periods, respectively 43, 36, and 33%. However, his geometric mean curves (Fig. 7) indicate that the "gravid" group would contribute much more than the "pregravid" one towards the form of a curve in which the categories were not separated. These two groups do not differ in respect to mating immediately before the blood meal, which was suggested (Lumsden, 1952) as a factor which might be expected to delay the advent of young females until later in the night. They do differ, however, in respect to oviposition, which precedes the blood meal in most of the "gravids" but not in the "pregravids". Thomson (1940) has shown with *Anopheles minimus* that most of the oviposition takes place in the first third of the night so that a preoccupation with oviposition might be expected to delay the advent of the "gravids" to bite. A difference of this sort might possibly explain the different character of the two curves. Such an effect could also explain the difference between the two curves for *A. pambaensis* which was previously ascribed to the preponderance of young females at the breeding places, not away from them. Also, one must expect the distance between the oviposition sites and the site of the catch to be of importance in determining the time of biting.

We are at present far from understanding the cause or causes of the differences which have been described, but it is felt that studies devoted to periodic activities other than biting may well shed considerable light not only on these differences but also on the general problem of the mechanisms of determination of biting cycles.

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# Influence de certains Facteurs nutritionnels et hormonaux, à des Températures variables, sur la Croissance des Larves d'*Aedes aegypti*, *Aedes albopictus* et *Anopheles stephensi*

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## RÉSUMÉ<sup>1</sup>

Dans les expériences faites avec *Aedes aegypti*, *Aedes albopictus* et *Anopheles stephensi* on a employé les régimes suivants: Protéines totales du lait (I), Thyroxine (II), Vitamine B 12 (III), Déjections de cobaye (IV). Les témoins étaient nourris avec de la poudre de biscuit de chien et de la poudre de viande.

A 28°C., les larves d'*Aedes aegypti* se développent très rapidement avec (I): larves au 4<sup>e</sup> stade le 3<sup>e</sup> jour, nymphes au 4<sup>e</sup> jour et adultes au 5<sup>e</sup> jour contre 9, 10, et 13 jours, respectivement, chez les témoins. Puis, par ordre décroissant les résultats les meilleurs sont obtenus avec les régimes IV, II, et III. Les dimensions des larves le 3<sup>e</sup> jour sont 8, 3 mm (I) 7, 9 mm (IV) 7, 4 mm (II) 5 mm (III) et 3, 8 mm (Témoins).

A une température sensiblement identique les différents régimes sont remplacés par le mélange de (I), (II), et (III). Les temps de développement sont à peu près les mêmes que pour (I) dans la première série d'expériences, bien que les dimensions des larves soient légèrement inférieures.

A 25°C. il y a allongement des durées de développement pour (I) (5, 6 et 7 jours) et surtout pour les témoins (20, 24, et 27 jours). Les dimensions des larves sont augmentées pour (I) par rapport à la première série (8, 7 contre 8, 3 mm. en moyenne) alors qu'elles sont diminuées chez les témoins (3, 3 contre 3, 8 mm).

A 20° la durée de développement est encore allongée pour (I). Cependant le régime est encore favorable puisque l'évolution se fait en 10 jours jusqu'au stade nymphal, 13 jours jusqu'au stade adulte, et que la taille des larves s'accroît encore (9, 3 contre 8, 3 mm à 28°C).

Avec *Aedes albopictus* et *A. stephensi* les résultats sont sensiblement identiques avec le régime (I). Avec les autres régimes et à 20° le développement larvaire est très ralenti ou même arrêté au stade III ou même au stade II.

De nos expériences, il résulte que de tous les régimes essayés, ce sont les protéines totales du lait qui agissent le mieux sur la durée du développement et sur les dimensions des larves. A une température relativement élevée, les résultats avec les déjections de cobaye sont presque identiques. Mais l'emploi des protéines du lait semble surtout avantageux à des températures basses. C'est en effet le seul régime qui permette aux larves de se développer normalement et d'atteindre le stade nymphal.

## DISCUSSION

H. I. SCUDDER. At Savannah, Georgia, U.S.A., I have been able to increase our production of *Anopheles quadrimaculatus* 50% over dog biscuit feeding by using a mixture of 60% white wheat flour, 25% yeast powder, 15% dried skim milk, and 10% dried ox blood. However, by contrast, this was inferior to dog biscuit for *Aedes aegypti*. Was your "protéines du lait" dried skim milk?

H. GALLIARD. I think protéines du lait is whole milk extract.

N.-M. COMEAU. Doit-on conclure que les facteurs température sont aussi importants que les extraits utilisés quant au développement jusqu'au 4<sup>e</sup> stade larvaire seulement?

H. GALLIARD. Le facteur température est prépondérant quand on utilise un milieu peu nutritif. L'hyperprotidine (extrait total du lait) a l'avantage de suppléer au facteur défavorable constitué par cet abaissement sans provoquer une putréfaction trop rapide du milieu.

R. LEVI-CASTILLO. Avez-vous fait une comparaison du milieu et de l'eau du biotope pour connaître de la différence? Sont des adultes ainsi obtenus aussi plus grands que des adultes normaux?

<sup>1</sup> A été publié dans *Annales de Parasitologie Humaine et Comparée* 32 (5-6). 1957.

H. GALLIARD. Il m'était difficile de faire une telle comparaison avec *Aedes aegypti* dans les conditions où je travaillais à Paris. Mais en Extrême Orient j'ai constaté les grandes différences biologiques existant entre une souche élevée au laboratoire et celles que l'on étudie dans leur milieu. Les adultes obtenus avec un régime d'hyperprotidine sont plus grands.

G. LUPASCU. (a) Si la fertilité de ces adultes n'est pas modifiée? (b) Si les larves de premier stade sorties de ces adultes sont plus grandes que les témoins?

H. GALLIARD. (a) La fertilité des adultes ne semble pas avoir été modifiée de façon significative. Des expériences faites en Extrême Orient, dans les conditions naturelles, j'avais tiré la conclusion que le régime alimentaire de la larve n'avait fort effet sur l'importance de la ponte à condition que la larve ait été placée dans des conditions moyennes. (b) Les larves au premier stade au moment de l'éclosion ont toutes la même taille. Les différences ultérieures dépendront du milieu nutritif.



# Some Nutritional Factors in Egg Production by *Aedes aegypti*

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## ABSTRACT

Egg production in an *Aedes aegypti* stock colony has been maintained by feeding the mosquitoes citrated, hemolyzed beef blood plus 10% honey from a saturated cotton pad. A subcolony fed a solution of albumin and honey is now in the 11th generation. Mosquitoes fed skimmed milk and honey were found to develop a curd in the ventral diverticulum after several days, and some of the factors causing curd formation have been determined. By supplementing various animal bloods with isoleucine, this amino acid has been shown to be a factor which results in the oviposition of different numbers of eggs by *Ae. aegypti* when fed on different host animals. The amino acid requirements for reproduction in *Aedes* have been found, the essential acids determined, and an optimum mixture of these acids developed. To study the amino acid diets further and to determine the vitamin requirements of the females of this species, a technique has been worked out to rear the larvae and feed adult mosquitoes under aseptic conditions.

My associate Mr. Dimond (1958) was invited to discuss the nutritional requirements for reproduction in insects at this Congress, and in his paper he has mentioned some of our studies on the nutritional requirements for egg production in *Aedes aegypti*. At the risk of being somewhat repetitious, I would like to begin by very briefly summarizing some of our previous work.

We first tried mixtures of beef blood and honey on saturated cotton pads to feed our stock colony of *Aedes aegypti* in order to maintain egg production. We found that the females would feed on a mixture of whole blood and 10% honey or sucrose. Later, for storage purposes, we froze several gallons of citrated beef blood. Freezing, of course, hemolyzed the blood but the females would feed on it if 10% sugar was added.

Next, we thought it might be possible to substitute something else for the blood, mix it with honey and still induce oviposition. We had some skimmed milk on hand, and we fed skimmed milk and 10% honey to *Aedes* females and obtained viable eggs. We then tried a number of proteins or hydrolysates of proteins and continued to get eggs with most of them. Egg albumin gave the highest egg production of any of the blood substitutes tested. We have been using 10 gm. egg albumin, 10 ml. honey, and 90 ml. water to maintain a subcolony of our *Aedes* stock. The egg albumin solution is the only source of food for the adults, and we have carried this egg albumin subcolony through 11 generations on this diet without any decline in the oviposition rate as compared with the parent generation. Egg albumin is even more convenient to use than the preserved blood, and egg production is equal to that on hemolyzed beef blood, although both are lower than natural feeding on a live host.

Finally, we fed mixtures of amino acids and sugar to female *Aedes* and had them lay eggs. Using the omission technique, we determined which amino acids were essential for egg development. The standard medium which was developed, given in Table I, included the ten essential acids plus cysteine and glutamic acid. Dosage levels of each amino acid were run on an original mixture, and the level of each acid giving the highest egg production was used to prepare a new standard medium. Then, the process was repeated, four times in all, until we had as nearly an optimum and balanced adult diet as possible.

The salt mixture itself is not essential for egg development, but salts are stimulatory, egg production being reduced by about one half when the salt mixture is omitted. With the omission of the sugars, no eggs were laid in repeated tests. The sugars in the test diets serve as an attractant, and the females take more liquid when sugar is present than when it is omitted. However, we cannot tell from these experiments whether sugars are nutritional requirements for reproduction.

One interesting side line came up in connection with *Aedes* females fed milk. We tried to set up a milk-fed subcolony, but egg production was highly variable and the females often stopped ovipositing after a week or 10 days. Several workers have shown that the

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TABLE I. Chemically Defined Diet Fed Female *Aedes aegypti*.\*

Component	Grams/100 ml.
L Arginine	0.38
L Cystine	0.15
L Glutamic Acid	1.00
L Histidine	0.15
DL Isoleucine	0.50
L Leucine	0.75
L Lysine	0.75
L Methionine	0.15
DL Phenylalanine	1.20
DL Threonine	0.30
L Tryptophane	0.30
DL Valine	1.00
Glucose	5.00
Fructose	5.00
Wesson Salt Mixture	0.15
Distilled Water	100 ml

\*In preparing this medium for use in the aseptic rearing technique, the amino acid components were used at a concentration of one half that given, and 0.15 gm. cysteine substituted for cystine.

destination of any food containing honey, other than whole blood and honey, is primarily the ventral diverticulum. On dissection of the milk-fed females, we found that the diverticulum contained a solid "milk curd". This curd increased in size during the feeding period, so that the diverticulum became large and distended. With a food such as egg albumin which does not curd, it is assumed that the food is pumped from the diverticulum to the stomach where it is digested. No digestive enzymes have been reported present in the diverticulum, thus, the milk, once it curds, apparently cannot be digested, but remains and blocks further intake of food to the diverticulum. The curd was tested and found to be casein. Tests also showed it was acid precipitated rather than enzyme precipitated. The final problem was determining whether the diverticulum itself was acid enough to cause the curding or whether bacteria in the milk were responsible. The pH of the diverticulum empty is between pH 5 and 6, which is above the isoelectric point of casein. However, for the first four days after emergence the mosquitoes are given only a 10% sucrose solution. We found that this sugar solution sometimes became very acid, as low as pH 2, and the diverticulum became correspondingly acid. This undoubtedly accounts for the fact that curding of ingested milk frequently commenced within one hour. At other times, curd formation might not begin for several days, presumably when the acid producing milk bacteria had lowered the pH sufficiently. As a test of the role of bacteria in curd formation, *Aedes* larvae were reared on a sterile diet under aseptic conditions, the emerging adults were kept under aseptic conditions, and the females fed only on sterile milk and honey. Upon dissection, although many were engorged, none of the females were found to have any trace of curd in the diverticulum even after 14 days. Thus, if the mosquito's diverticulum does not become acid before feeding on milk, bacterial action is probably the primary factor in curd formation.

Another factor we have been investigating, follows the work of Woke (1937) in which he showed that *Aedes aegypti*, fed on different hosts, laid fewer eggs when fed on man or monkey, than when fed on rabbit, guinea pig, canary, frog, or turtle. More recently, Greenberg (1951) conducted some feeding experiments with *Aedes aegypti*. His technique depended on the mosquitoes piercing a membrane to feed, but he was unable to get them to take any liquids unless washed sheep erythrocytes were added to the medium. A few eggs were laid when females were fed the washed erythrocytes alone. He also found that supplementation of the washed erythrocytes with the amino acid isoleucine increased egg production. A mixture of nine other essential amino acids did not have any effect on egg production. We noted this effect of isoleucine on egg production in our early feeding experiments when we used either commercially prepared beef hemoglobin or beef blood. Once we had determined that protein and not blood *per se* was the requirement for reproduction and had found which amino acids were required in the diet of the female, we

decided to look into the relationship between different bloods and egg development in *Aedes* feeding on these bloods.

The results of these studies are given in Table II and are based on the number of eggs laid per female per mg. of blood taken. In the column titled "bite", groups of mosquitoes of known unfed weight were allowed to feed on the animals, then re-weighed, and held for egg counts. There is a significant difference in the number of eggs laid by those which fed on man or sheep as compared to those fed on pig or rabbit. The same holds true when the mosquitoes fed from saturated cotton pads on the respective bloods which had been hemolyzed and mixed with 10% honey (Table II, second column, 0 mg. isoleucine added).

TABLE II. Relationship Between Bloods with Different Isoleucine Contents and Egg Production by *Aedes aegypti*.

Host Blood	Bite	No. eggs per female per mg. blood taken				Isoleucine content of hemoglobin or globin in gm/16 gm N**
		Mg. isoleucine added per ml. blood*				
		0	0.75	2.50	6.00	
Man	26.7	25.1	28.8	32.1	31.5	0.1
Beef	—	25.5	29.9	32.5	30.7	< 0.1
Sheep	29.5	26.9	28.3	31.3	35.9	0.3
Pig	37.3	32.7	30.0	—	33.1	1.6
Rabbit	38.5	33.3	34.1	—	34.4	1.7

\*Hemolyzed blood plus 10% honey fed from cotton pad.

\*\*Data taken from Block and Bolling, "The Amino Acid Composition of Proteins and Foods".

An inspection of the data in the monograph of Block and Bolling (1951) revealed that the level of each of the essential amino acids in the hemoglobin or globin of the different animals was nearly the same except for isoleucine. This isoleucine deficiency in blood protein has been confirmed in feeding tests by various investigators. Therefore, we supplemented each of the bloods with different amounts of isoleucine. In the case of rabbit and pig there was no significant increase, but oviposition from the three bloods normally low in isoleucine were raised by the addition of isoleucine, with the result that egg production was nearly equal in all the bloods. The addition of nine other essential acids had no effect on the oviposition from these bloods.

There is still more work which we hope to do on this problem, particularly with other bloods, but we feel that we now have good evidence that the nutritional differences in bloods may affect the reproduction of the female mosquito.

For the last point which I want to discuss, I would like to return to our studies of the nutritional requirements for reproduction using chemically defined diets. As yet, I have not mentioned the role or importance of vitamins, which would be the next logical subject to investigate after the amino acids. The vitamins, however, could not be studied in open cages as were the amino acids, because of the factor of contamination by microorganisms. I have been developing a method by which larvae are reared aseptically, on a lactalbumin-yeast diet, either in a sterile gallon jar into which the adults emerge directly, or separately in a sterile flask from which the pupae were taken, rinsed with sterile water, and transferred to a sterile gallon jar for emergence. The adults can then be fed chemically defined test diets and held for oviposition in the sterile adult jars. The object of the pupal rinse was to remove as completely as possible any larval food which might serve as a source of vitamins for the adults. The adult jars were set up, before autoclaving, with the adult test diet, an oviposition container, and tubes for drawing off samples of the adult diet and oviposition water into bottles of nutrient broth, in order to test the sterility of the jar at the end of the experiment.

Using the same amino acids as in Table I, female *Aedes aegypti* developed and laid eggs when no vitamins were added to the adult test diet. We have not had the opportunity as yet to observe whether, if included in the diet, vitamins will stimulate greater egg production. We have repeated the tests in which each amino acid was omitted singly from the diet and have found no difference in the requirements of essential acids for egg production.

From the standpoint of contamination, the pupal transfer is the weakest point in the technique, but I have modifications in mind which should simplify it and eliminate this source of contamination. Much of the contamination problem may also be overcome by extending the autoclaving period from one to two hours.

We hope eventually to replace the Brewer's yeast and lactalbumin larval medium with a chemically defined diet which I have been developing (Lea 1958). Thus, using adults reared from larvae on such a medium and feeding the adults known mixtures of amino acids, we hope to run the entire life cycle from egg to egg under sterile conditions. The *Aedes* mosquito with its specific nutritional requirements for reproduction make it an excellent insect in which to study the effects of larval nutrition on the adult requirements for egg development.

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# Oviposition Activity of Mosquitoes in the Laboratory (Diptera: Culicidae)

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## ABSTRACT

*The elimination of determinate error in oviposition site selection experiments and nutritional studies of mosquito reproduction in the laboratory depends upon understanding the environmental and physiological factors which effect the oviposition response of mosquitoes. Several critical factors are discussed. Three species of Anopheles were studied to determine selection among fresh water and saline oviposition sites. Although A. quadrimaculatus, A. freeborni, and A. aztecus laid more eggs on distilled water than on any saline concentration, their eggs were collected over a wide range of concentrations due to environmental factors. Separation of test sites by sufficient distance to prevent accidental overlapping distribution of eggs oviposited from flight confirmed the preference for fresh water. Egg development and the subsequent oviposition response of Aedes aegypti were studied in relation to the blood meal under various conditions. The oviposition response was affected by isolation of females and mating, and it was determined that ovarian development beyond Christopher's State II was a more reliable measure of the efficiency of a blood meal than was the oviposition response.*

Recent studies have emphasized that an understanding of the factors which affect the oviposition response is necessary in experimental work on site-selection and nutritional studies with mosquitoes. Factors affecting the selection of aquatic oviposition sites are of interest in the case of specific disease vectors. The oviposition response has also been utilized as an index of the efficiency of test solutions in study of the dietary component in the mosquito's blood meal which stimulates egg development. In each of these lines of work, critical data has been provided by the mosquito ovipositing in the laboratory under a highly artificial environment. It is the purpose of this report to discuss certain of the factors which can alter the oviposition response and cause variation in the results.

While field observations strongly supported the premise that restriction of certain species to a particular type of larval breeding place was a result of selective oviposition activity on the part of the adult female, laboratory experiments designed to study selectivity yielded conflicting results. Such results came primarily from experiments with *Anopheles*, in which attempts were made to demonstrate a preference among a series of test sites differentiated only by saline content of the water. The procedure has been one of introducing a series of solutions in containers into the cage. These were shifted around to avoid any possibility of a "position preference" for any one location influencing egg distribution. This produced clear-cut results with species of *Aedes* (Wallis, 1954a) and *Culex* (De Zulueta, 1950). However, in testing *Anopheles*, a shifting only served to give each container an equal chance of catching eggs oviposited from flight. These eggs accidentally overlapped from the preferred sites onto solutions not selected. The amount of this overlapping depended upon the species of *Anopheles* used in the test because of the differences in their flight patterns. Those which exhibited predominantly up-and-down movements over a restricted area produced less overlapping than those which ranged horizontally over a considerable area. In the case of the former, relatively small increases in distance between test sites greatly reduced the per cent of overlapping and resulted in more clear-cut demonstrations of selective egg deposition (Wallis, 1955).

While the space factor was important in obtaining valid results from *Anopheles*, it was of little consequence in experiments with *Culex* and *Aedes* which oviposited while in contact with the oviposition site. Instead, the site chosen by these depended upon the series of solutions available in the testing environment (Wallis, 1954b). In laboratory tests of *Aedes aegypti*, marked variation was obtained in the distribution of eggs among test sites when different combinations of solutions were offered. If given only three solutions: distilled water, one, two, and three per cent NaCl solutions, the mosquito would choose distilled water. However, when an adequate number of solutions was provided, ranging from distilled water up to one per cent NaCl, the maximum oviposition occurred on .125 per cent NaCl rather than on distilled water.

In an altogether different line of investigation concerning the nutritional factor provided in blood which stimulates ovarian development in mosquitoes, numerous investigators have used the oviposition response as an assay to evaluate test diets. However, results of recent work show that caution must be exercised in the use of such an index. Woke (1955) demonstrated deferred oviposition with *Aedes aegypti*. Gillett (1955) has shown that one strain of *Aedes aegypti* will not lay eggs unless mating has occurred. Similar results were obtained by Lang (1955) with the same species and a more uniform response was obtained from mated mosquitoes than from virgins.

To determine whether mating played a role in stimulating egg formation and the extent of the effect on the oviposition response, tests were conducted on series of mated and virgin blood-fed *Aedes aegypti*. The results show that oviposition time of virgin females was longer and more variable than that of mated mosquitoes. In addition, 28 per cent of the virgins retained eggs after 14 days. The incidence of retention was approximately three times greater than in the mated females.

Of these females retaining eggs, the number retained was compared with the number oviposited. The sum of these two counts provided an index of the number formed. Approximately seventy per cent of the eggs formed in the virgins were retained. With mating affecting the oviposition response to this extent, it would seem that a criterion for activity of a test feeding should be based upon the number of eggs formed at a fixed time after the feeding.

Dissection of mated and virgin females at 24-hour intervals after a blood meal revealed little influence of mating on ovarian development. Rapid changes took place within three days post-blood meal in individuals of both the virgin and mated groups. In comparison, no such activity appeared in females engorged only on sucrose. It is possible, therefore, to evaluate a test diet by examination of dissected ovaries two to three days after administration of the test preparation. This provides a rapid assay devoid of the variation associated with the oviposition response.

In conclusion, the laboratory cage provides a somewhat restricted and highly simplified environment for experimental work with mosquitoes, and with each species and in each type of investigation, there must be particular care exercised to control critical environmental conditions which can contribute excessive variation to the results.

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## DISCUSSION

R. LEVI-CASTILLO. Does the range of solutions affect the oviposition rate of the mosquitoes and the viability of the same? Does it change the number and the pattern of the eggs?

R. C. WALLIS. The oviposition response on repellent solutions in higher per cent solutions is restricted and more eggs are retained until a more acceptable solution is encountered. Oviposition of some eggs is on concentrations which are unsuitable for hatching and young larvae, and low viability results.

W. H. R. LUMSDEN. Has Dr. Wallis any information on the periodicity of oviposition by mosquitoes in these experiments?

R. C. WALLIS. Yes. However, experiments designed to investigate this have not been conducted. Casual observations however reveal bimodal periodicity—early morning and late afternoon for *Aedes aegypti*.

# Orally Administered Insect Repellents: Approaches and Problems Related to the Search

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An active search for an insect repellent which could be administered orally has been progressing for several years. This work which was made possible by a grant from the Defence Research Board of Canada, is being conducted by the Ontario Veterinary College where accommodation is suitable for experimental animals.

The knowledge that some medicines and other substances are eliminated slowly through the skin or with the breath suggested the possibility of a systemic, orally-administered repellent. The pungent odour of hexachlorethane, for example, may be smelt in the breath of cattle several days after they have been treated with this drug for liver fluke disease. Another illustration is the characteristic and constant aroma of the habitual garlic eater. It seemed likely from this that the emanation of an insect repellent from the surface of the body for many hours, days, or even continuously would have endless possibilities. One principal advantage of a systemic over an externally applied repellent would be the elimination of repeated applications of oily, messy substances to the skin, with their associated irritating effect on the mucous membranes.

Briefly summarized, the work of our researchers in this field has included the administration to man or animals of all available substances which might reveal possibilities as orally-administered insect repellents. Each agent was introduced directly into the stomach of the experimental animal. However, in some instances, drugs, such as certain hormones, were necessarily administered by needle and syringe. The treated vertebrates were then exposed to mosquitoes for two or more days. Yellow fever mosquitoes or native species were used to check the effects of treatments. Test animals included guinea pigs, rabbits, rats, mice, hamsters and occasionally man.

The first substances tested were internal doses of every available insect repellent. In each case, as large a dose as was proved tolerable was given. A year or so later we tested many volatile oils on the assumption that good repellents are volatile compounds. Then followed a series of tests with sex and other hormones, selected alcohols, aldehydes, ethers, esters, acids, amides and phenols. We also tried other hydrocarbons, some alkaloids, ketones, oleo resins and salts, body deodorants and antihistamines. Miscellaneous tests on substances, alleged to keep humans free from insect attack, included the taking of vitamins in daily doses, the drinking of copious quantities of coffee and soya bean oil, and the eating of the odoriferous fruits of the ginkgo tree.

Our approach to this problem so far has been mostly empiric on the possibility that it might lead to results through a short cut. To this date we have had no success. In contrast to the empirical approach, a reasoned one would call for greater knowledge of the factors which attract or repel insects than is presently available. Also, we need to know more about vertebrate skin physiology and the nature and fate of substances administered internally from the time they reach the stomach to their chemical state on elimination through the skin.

One of the problems that had to be considered in comparative attraction tests was the possible rôle of biological variations in our test animals, such as age, sex and physiological condition (pregnancy, diet and blood sugar levels). For two years much time was spent studying these variables but none of them was found to make any significant difference in the attractiveness of animals, at least not to yellow fever mosquitoes.

We have always felt that the repellent action of the drugs tested may be limited inasmuch as small laboratory animals do not perspire as freely as large animals. However, we have little choice in our use of small animals because large animals are not economically practical for use in extensive tests. Experimental tests on man can only be done when the substances being tested are known to be safe. Incidentally, a time-consuming problem involved in this work is that of establishing safe dosage when the toxicity of a substance is not known. This question applied to many of the agents tested and to be tested as possible repellents for oral administration.

Various other problems confront the researcher in projects of this kind, chief amongst which is the maintenance of experimental animal colonies and mosquito colonies. Health, temperature, humidity and other similar matters all require constant consideration. The activity of mosquitoes varies with their age and from day to day. Consequently all experiments must be replicated, and position correction factors must be constantly considered when exposing treated animals to the insects.

In conclusion, may I suggest that future research in this area will be associated with better understanding of the physical and biochemical mechanisms which attract insects to their blood meals. This paper is presented with the hope that it may inspire others to join in the search for an effective systemic insect repellent.

### DISCUSSION

W. H. R. LUMSDEN. In catches of mosquitoes on cows in Entebbe, Uganda, mosquitoes were taken on recumbent sick cows but not, on another occasion, on a recumbent cow anaesthetized with chloral hydrate.

A. A. KINGSCOTE. We have not used cattle in any of our tests but will certainly examine the possibilities of chloral hydrate.



# The Genus *Culicoides* (Diptera: Ceratopogonidae) in Canada; an Introductory Review<sup>1</sup>

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## ABSTRACT

More than 35 species of *Culicoides*, including a number of undescribed forms, have been found in Canada, and it is believed that many more remain to be discovered. The group occurs in damp places throughout the country and extends onto the tundra as far north as Victoria Island. Following a general account of the bionomics of the genus, the salient features of the regional faunas are discussed. Some of the sub-arctic species show affinities with those of the Old World; nevertheless only *C. obsoletus* (Meigen) and *C. chiopterus* (Meigen), both from more southerly regions, are as yet recognised in both the palaearctic and nearctic regions. The sub-arctic and the prairie species tend to lose the blood-sucking habit, and these autogenous forms occur in great abundance where the soil provides a rich larval habitat. The blood-sucking species feed on man, other mammals, and birds. *C. obsoletus* is a widespread pest especially in Quebec and Ontario, as is *C. yukonensis* Hoffman and others in Western Canada. *C. variipennis* (Coquillett), the vector (in U.S.A.) of blue-tongue of sheep, is abundant in Alberta and British Columbia. The occurrence in Canada of two species of the related genus *Leptoconops* is noted.

## INTRODUCTION

*Culicoides* is a large and almost world-wide genus of small biting flies which until recently has been comparatively little studied and the medical and veterinary significance of which is perhaps only gradually becoming apparent. In these respects, the Canadian fauna is no exception. In his recent review Fox (1955) listed 51 species in the United States, this number itself comparing unfavourably with the 35 known in Britain, but only 10 in Canada. With the exception of a few records of blood sucking, almost nothing is known of their habits and life-histories.

I have recently been able to study these insects in several provinces, and, with assistance from colleagues, have assembled a collection of 35 or more species, a considerable number of them apparently undescribed. I shall attempt to give a preliminary account of some of the general features of this fauna, together with an outline of the bionomics of the genus.

## BIONOMICS

The eggs of *Culicoides* (Parker, 1950; Jobling, 1953) cannot resist desiccation and probably are always laid on a moist substrate. Typically, they develop rapidly and hatch within a few days or weeks, but the egg stage of *C. vexans* (Staeger) lasts for several months and another European species (*C. grisescens* Edwards) overwinters in this condition. The larvae (Dorsey, 1947; Hill, 1947; Kettle and Lawson, 1952; Mayer, 1934a, b; 1955; Megahed, 1956; Nicholas, 1953; Williams, 1951; and references cited in these papers) likewise live in a wet habitat; but when this has been said, it seems difficult to make further generalization.

Predominantly, perhaps, they are to be found in permanently marshy soil around the margins of pools and lakes, or in damp pasture and wet peaty areas. Many species are characteristic of coastal salt marshes and others of the margins of alkaline pools in inland areas. Several occur in farmyard soil rich in organic matter and a few live in the dung of cattle and, perhaps, of poultry. Accumulations of humus and leaf-refuse in tree holes and the axils of large leaves, and the rotting stems of fibrous and woody plants are inhabited by a number of species. The larvae live mainly in the upper layers of these moist or semi-solid materials; if the habitat becomes flooded, they swim with a rapid side to side lashing movement, but for short periods only except in a few species. They are probably unselective in their feeding, and the gut contents often consist of a mixture of organic fragments and soil particles; in the laboratory it may be useful to improve the medium by exposing the culture dishes to light to promote the growth of algae, or by adding a small amount of yeast.

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There are four larval instars. In temperate regions the winter is passed as a well-grown larva and development is completed in the spring. The larvae come to the surface to pupate, and the pupae drown unless the spiracles (on the prothoracic horns) have access to the air (Weerekoon, 1953). If the larvae pupate at the surface of moist soil, as they often do, and the area then becomes flooded, the pupae float on the surface alongside emergent vegetation and can then be readily collected. The pupal period is short, seldom more than two weeks. Many species have only a single generation in the year whereas others produce one or more additional generations during the summer. In tropical areas there may be a succession of generations almost throughout the year, the full life-cycle occupying a period of about a month.

Mating (Downes, 1955), in the typical case, takes place in flight. The males form swarms over conspicuous landmarks, and the females fly into these swarms and are captured almost immediately. A few species, however, do not require this flight and will mate readily in small containers in the laboratory. Both sexes visit flowers for nectar, but only a few observations have been reported (Mayer, 1933). The females are notorious biting pests of man and livestock, and in a few instances, of birds, e.g., *C. arakawai* Arakawa (see Arnaud, 1956); but even in this conspicuous aspect of their bionomics nothing is known of the majority of species. The lesion produced by the bite has recently been described by Arean and Fox (1955). The blood-meal (Downes, 1950, Jobling, 1953, and many other authors) initiates the development of the ovaries and after a few days the eggs, usually between 50 and 200 in number, are laid in a loosely scattered group. One blood-meal is sufficient, normally, for the full development of a group of eggs. It is relatively uncommon to encounter females with eggs in any intermediate stage of development and it seems that after the blood meal they become inactive until the time of the oviposition flight. It has generally proved difficult to maintain the adults in captivity for long periods, but *C. nubeculosus* (Meigen) will live for more than a month and is able to complete as many as five ovarian cycles, each depending on a new blood-meal. Among the principal requirements for longevity are a readily discoverable source of sugar and a rather high atmospheric humidity.

The adults (Downes, 1950; Foote and Pratt, 1954; Hill, 1947; Nicholas, 1953; Parker, 1949; Sailer, Marks and Lienk, 1956; and references cited) usually fly in the evening and, in certain species at least, again after sunrise. A few species are on the wing throughout the night, and a few others in the mid-day sunshine. Several of the crepuscular forms bite during the day in heavily shaded situations, an observation that suggests that light intensity is an important factor in the control of activity. The insects often continue to enter light traps for a large part of the night (e.g., Williams, 1955), but the light may have a stimulating effect and it is doubtful if this represents a natural pattern of activity. The period of flight is not always identical in the two sexes, indicating perhaps that mating and the hunting of prey take place at slightly different times; there is evidence also that oviposition is confined to a rather sharply limited period. The flight of *Culicoides* is slow and occurs only in calm conditions; it ceases altogether when the wind reaches a speed of about 3 m.p.h. Related to these facts, perhaps, is the very limited range of flight of most species; detailed experiments on *C. impunctatus* Goetghebuer have shown that it flies, on the average, only about 80 yards from the larval habitat (Kettle, 1951) and general observation indicates that this is substantially true of many others also. *C. tristriatulus* Hoffman and *C. peleliouensis* Tokunaga, which may be found several miles from their breeding grounds, are exceptions to this rule (Williams, 1951; Dorsey, 1947.) The adults are not often observed at rest; they appear to hide during the day in crevices in the ground or in low-growing vegetation (Jobling, 1953; Sailer *et al.*, 1956).

#### AFFINITIES OF THE CANADIAN FAUNA

Many species being undescribed, or at least not yet identified, it is scarcely possible to give a detailed account of the affinities of the fauna. All except one of the identified species are to be found also in the United States, some of them having a very wide distribution. The exception, perhaps more apparent than real, is *C. gigas* Root and Hoffman, a western species which, although hitherto little known, is widespread and abundant in the Prairie Provinces and in British Columbia.

Two species only are recognised with certainty both in the Nearctic and in the Palaearctic regions. *C. obsoletus* (Meigen) is found over much of the United States and in the

southern parts of Canada from Labrador to British Columbia; it is also widespread in Europe and extends, apparently, to Japan. *C. chiopterus* (Meigen) is recorded from Maryland and has been found also in other eastern localities, including the neighbourhood of Ottawa; in the Old World it is known from Austria northwards to Scotland and in Russia. Both these species are often associated with man; *C. obsoletus* is common in farming areas, the larvae live in leafy humus, and the females readily feed on man and domestic animals; and the larvae of *C. chiopterus* live in cow dung. It is possible, therefore, that they may from time to time have been introduced from Europe.

Of the unidentified forms, some are likely to be Canadian species of restricted range whereas others will no doubt be found to have a wide distribution. The existence of a distinctive northern fauna has been clearly established. At Churchill, Manitoba, which lies on the southern limits of the tundra, nine species have been found and of these seven seem to be undescribed; other new species have been detected at Whitehorse, in the Yukon, and at Goose Bay, in Labrador. Several of those from Churchill are closely related to northern European forms, yet none of them, it seems, are conspecific. Two at least are true arctic species extending far into the tundra region, to Coral Harbour on Southampton Island and to Cambridge Bay on Victoria Island (lat. 69°N), respectively. Possibly some of these northern forms will in due course be shown to have a circumpolar distribution.

### REGIONAL CONSIDERATIONS

It is likely that members of this genus occur in all parts of mainland Canada where suitable larval habitats exist, and that, in spite of recent work, a large fraction of the fauna yet remains to be discovered. It is not the purpose of this section to give a systematic account of the species and their distribution as now known, but merely to bring out the general features of the fauna and to comment on certain matters of interest.

It may be said at the outset that nothing is known of the coastal salt-marsh species, although several must occur both on the Atlantic and the Pacific coasts. In the United States and elsewhere, many salt-marsh species are important biting pests.

In the mixed deciduous forest of the St. Lawrence-Great Lakes area, now largely cleared for farming, *C. obsoletus*, *C. biguttatus* (Coquillett) and *C. crepuscularis* Malloch are among the more abundant species. As might be expected from their distribution in the United States *C. travisi* Vargas, *C. stellifer* (Coquillett) and *C. haematopotus* Malloch (the latter biting man) are also to be found, although less commonly. *C. chiopterus* occurs in small numbers, breeding, as it does in Europe, in cow dung. Little is known of the details of the life cycles, but it is suspected that many species in this area have two or more generations in the year. Some 12 species occur in the neighbourhood of Ottawa.

Contrary to experience in parts of the United States (Edmunds and Keener, 1954), *C. crepuscularis* has not been recorded as a biter of man. This species is widespread in southern Canada, occurring also in the prairies and in British Columbia, and it exists in a variety of local forms the taxonomic status of which has not as yet been investigated. *C. biguttatus* is equally widespread and extends also into the southern parts of the coniferous forest. Previously recorded as biting man (Metcalf and Sanderson, 1931), it has been found on a farm in Quebec feeding also on horses, cattle, and domestic fowl. Jellison and Philip (1933) found engorged females in the nest of a crow (and also *C. crepuscularis* in the nest of a magpie) and Judd (1954) has taken it in the nest of a catbird; to these records may now be added a series from the nest of a kingbird (Osgoode, Ontario, 17 June 1952, R. de Ruette). Recently another bird-biting species has been detected (Fallis, 1957) feeding on ducks in Algonquin Park and acting as the vector of *Haemoproteus*; it appears to be undescribed.

*Culicoides obsoletus*, as foreshadowed by Twinn (1931), is a serious biting pest of man, certainly by far the most important species in Eastern Canada. It is abundant especially in the southern parts of the conifer forest from the Gaspé Peninsula to Georgian Bay, and from time to time, but usually, it seems, only for a few days in each season, it may become an almost intolerable problem in lumber and tourist areas. It also attacks cattle, horses, and domestic fowl. It has been reared on occasion, principally from decaying leaf refuse in marshy woodland sites, but although it is so common and widespread in both the Old and the New World, the larvae have never been obtained in large numbers and their characteristic habitat remains undefined. The species probably extends for a considerable distance into



the boreal forest, and at Goose Bay, Labrador, it again appears to be the commonest member of the genus.

The northern fauna, as already indicated, is extensive. Of the nine species found in the vicinity of Churchill, four occur mainly in the forest areas a few miles to the south of the town and an equal number predominantly on the open tundra. The larvae of three of the tundra species are exceedingly abundant in the soil around shallow pools (Fig. 1), locally attaining populations of nearly one-thousand to the square foot. It seemed impossible that

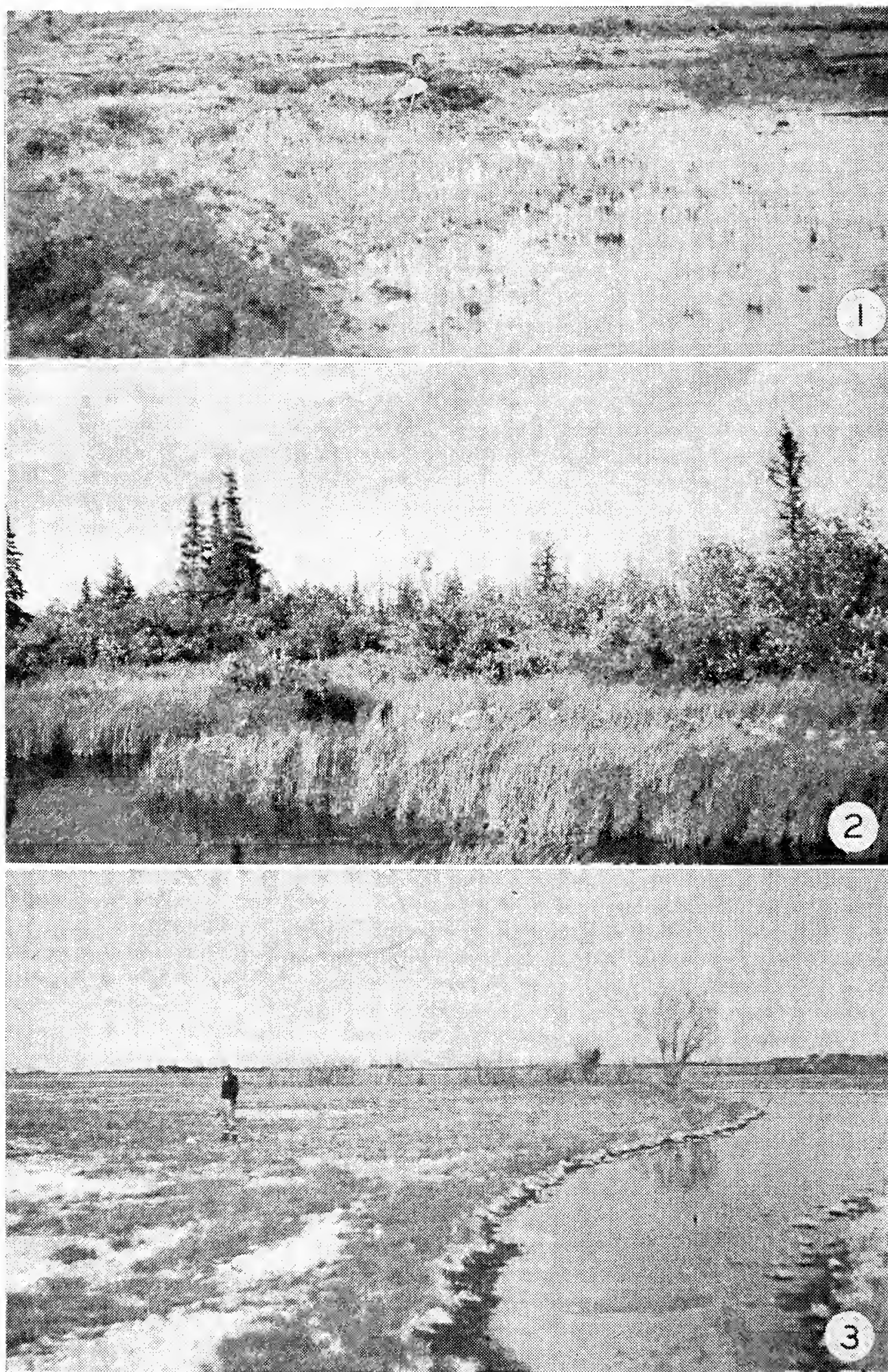


Fig. 1. Tundra pool, Churchill, Manitoba. Larval habitat of three autogenous species of *Culicoides*. Fig. 2. Marsh in boreal forest, with *Cicuta*; Churchill, Man. Adult feeding ground of *C. sp. near yukonensis*; probable breeding ground of this and two other species. Fig. 3. Alkaline slough, Brooks, Alberta. *C. gigas* oviposits on the black soil of pool margin. Larval habitat of three species.



the females of species occurring in such numbers could depend upon a supply of vertebrate blood, and in each case it was found that the eggs began to develop immediately on emergence from the pupa and ripened within two or three days, without any food having been taken. This, the first reported instance of *autogeny* in the genus *Culicoides*, may reasonably be interpreted as an adaptation to arctic conditions, rich in food for the larvae but severe for a blood-sucking adult with limited powers of flight. One of these forms, perhaps a local race of *C. alaskensis* Wirth, is also able to mate on contact, that is without a mating flight; it appears to be of very sedentary habits and the adults were rarely found except by sweeping vegetation in the neighbourhood of the breeding grounds.

The females of two species, *C. gigas* and *C. sp. near yukonensis*, frequently bite both man and dogs; the blood-sucking habits of the remainder are unknown. Both males and females of the last-mentioned species also feed with some regularity on the nectar of *Cicuta* and *Heracleum* (Umbelliferae), in part just before and in part during the period of the evening flight (Fig. 2). Again the nectar source, if any, of the remaining species is unknown.

The evidence obtained by sampling the larvae at intervals during the season suggests that at least two of the species occurring at Churchill have a life cycle of two years, with the first winter spent as a young, and the second as a full-grown, larva.

The prairie fauna is also extensive, and again includes a number of undescribed species. Larvae of several species are abundant around the margins of sloughs, often of high salt content (Fig. 3). Many of these sloughs dry up during the summer months and it is evident that they provide only a temporary habitat which must frequently have to be re-colonized; but in irrigated areas the situation tends to be somewhat more stable. At Brooks and Macleod, in southern Alberta, repeating the experience at Churchill, four autogenous species were discovered; and these four also produced very high populations. Perhaps in the prairies there is commonly a sufficiency of rich larval habitats together with a scarcity, or only occasional abundance, of vertebrate hosts; certainly, moreover, flight of the adults is severely restricted by the prevailing windy conditions.

In *Culicoides gigas* the first ovarian cycle is completed without a blood meal, but since (at Brooks) the species also bites man very readily, it seems probable that it then proceeds to a second ovarian cycle which depends on this blood meal, thus resembling *Culex molestus* Forskål among the mosquitoes. A very distinctive oviposition flight takes place shortly before sunset, during which the insects fly along a narrow zone of the margin of the pool at a height of a few inches only, ultimately alighting on the blackish soil to deposit their eggs (Fig. 3).

At some of the alkaline sloughs in southern Alberta, *C. gigas* is accompanied by the related *C. variipennis albertensis* Wirth and Jones and by a new species of the same group. The latter is not only autogenous but has markedly reduced mouthparts. As briefly recorded in another paper presented at this Congress (Downes, 1958), its mating behaviour is also aberrant, and the species provides an interesting example of correlation of atypical features of structure, physiology, ecology, and behaviour, very much as does *Cnephia dacotensis* (Dyar and Shannon) among the black flies.

The fauna to the west of the Rocky Mountains is extensive and complex, as it is in so many other groups. *C. variipennis* is represented by two forms (sub-species *variipennis* (Coquillett) and *occidentalis* Wirth and Jones) and may occur in close association with livestock. This is a matter of some interest as it has recently been shown to act as the vector of blue-tongue, a virus disease of sheep, in Texas (Price and Hardy, 1954). *C. obsoletus* and *C. yukonensis* Hoffman are perhaps the most frequent man-biting species; the former is also a pest of cattle and horses (Hearle, 1938). The latter is a member of the group of species very closely related to *C. pulicaris* (L.) (see Downes and Kettle, 1952) and has been thought to be the only member of the group in North America, and to be restricted to the far West. The series of collections now under review, however, show that members of the *pulicaris* complex occur also in southern Alberta and Saskatchewan, at Churchill, Man., around Ottawa, and at Goose Bay, Labrador.

Many other species occur, but it would be premature to make more detailed comments. The *Culicoides* of British Columbia are now being studied by Mr. L. C. Curtis, of Kamloops.

In conclusion, a brief note may be added on the related genus *Leptoconops*. The genus is characteristic of warm, and indeed mainly tropical, regions; unlike *Culicoides* the adults tend to bite during the day, often in bright sunshine, and the larvae live in dry, probably

often sandy, environments (Smith and Lowe, 1948). Many species are important pests of man, of other mammals, and of poultry. The first to be detected in Canada was an undescribed form from the tundra at Churchill, Manitoba, constituting the most northerly record for the genus (cf. Gutsevich, 1945). Latterly, it has become clear that *L. kerteszi* var. *americanus* Carter, a locally important pest species in the western United States, occurs in the southern parts of the Prairie Provinces and also in British Columbia, where Curtis (1957) has reported that it attacks man.

## APPENDIX

The following list of 12 species of *Culicoides* known to have been recorded to date from Canada include also original references to the records and observations.

- C. obsoletus* (Meigen). British Columbia (Hoffman, 1925); Quebec (Twinn, 1931); Saskatchewan, Alberta, and British Columbia (Curtis, 1941); Alberta, British Columbia (Hearle, 1938).  
*C. cockerellii* (Coquillett). Alberta, Northern Territory (? = Northwest Territories) (Hoffman, 1925); British Columbia (Curtis, 1941).  
*C. yukonensis* Hoffman. Yukon (Hoffman, 1925); British Columbia (Curtis, 1941).  
*C. denningi* Foote and Pratt. Saskatchewan (Foote and Pratt, 1954).  
*C. jamesi* Fox. British Columbia (Wirth, 1952).  
*C. variipennis* (Coquillett). British Columbia (Curtis, 1941); Ontario, Alberta, British Columbia (Wirth and Jones, 1957).  
*C. gigas* Root and Hoffman. Saskatchewan (Root and Hoffman, 1937), British Columbia (Curtis, 1941; Leech, 1943).  
*C. unicolor* (Coquillett). Saskatchewan, British Columbia (Curtis, 1941).  
*C. crepuscularis* Malloch. Saskatchewan, British Columbia (Curtis, 1941).  
*C. sphagnumensis* Williams. Quebec (Williams, 1955).  
*C. biguttatus* (Coquillett). British Columbia (Curtis, 1941), Ontario (Judd, 1954).  
*C. travisi* Vargas. Ontario (Judd, 1957).

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## DISCUSSION

J. G. REMPEL. 1. Have you collected *Culicoides* in Saskatchewan? I am asking this question because of the large number of alkaline sloughs and alkaline lakes there. 2. How difficult is it to rear *Culicoides*?

J. A. DOWNES. 1. Mr. Fredeen and Mr. Shemanchuk have sent me considerable collections taken at light at Saskatoon and at Prince Albert. I presume there is an extensive prairie slough fauna in Saskatchewan, as in Alberta, but I have not collected in Saskatchewan myself. 2. It is rather easy to raise well-grown larvae by maintaining them in the soil of the original habitat at its natural dampness. But relatively few species have so far been reared from eggs.

W. E. SNOW. Do you have any species of *Culicoides* breeding in moist wood?

J. A. DOWNES. *Culicoides obsoletus* has been reared from moist rotting logs in England. I have not explored this habitat myself, but I think it might be profitable to do so.

C. B. PHILIP. Do you have any Canadian *Culicoides* in which there is an abundance of adults, but larvae scarce, suggesting breeding in drier situations than usual, such as are found in a few species of Tabanids breeding in fairly dry, non-aquatic situations? What proportion of Canadian species are known in the larval stage or have been reared?

J. A. DOWNES. I have reared some 20 species from well-grown larvae by maintaining them in samples of the natural habitat. I would not presume to say I am sure of the typical or major habitat of any but a fraction of these species, and it is possible that some may inhabit drier situations than are yet realized.

J. FRAGA DE AZEVEDO. At what time do the *Culicoides* that you studied bite? How was the blood in the gut of the *Culicoides* identified? How many generations have they in your country considering that it is a cold country and what is their duration?

J. A. DOWNES. The blood in the gut was identified by the precipitin test. My colleague Mr. A. E. R. Downe has built up a collection of anti-sera and very kindly made the tests. I believe the other questions have been dealt with in my paper, but it should be remembered that the species differ considerably.



# Seasonal Emergence of Black Flies from Streams in Northern Canada

By F. P. IDE<sup>1</sup>, C. R. TWINN<sup>2</sup>, AND D. M. DAVIES<sup>3</sup>

## ABSTRACT

The date of the beginning of emergence of black flies from streams was established for Churchill, Man., in 1948, 1949, and 1950, and for Algonquin Park, Ont., Baker Lake, N.W.T., Whitehorse, Y.T., and Goose Bay, Labrador, in 1949. At Churchill, for one location in a stream, the beginning of the black-fly season varied by two weeks between the years 1948, 1949, and 1950 and it has been calculated that this date would vary by over a month between the earliest and the latest seasons. A factor of 6 day's retardation between the latitudes of Algonquin Park, Ont., and Churchill, Man., and  $7\frac{1}{2}$  days between the latitudes of Churchill and Baker Lake, N.W.T., along the meridian of Churchill, gives a close approximation to the observed facts in 1949. Variation in the beginning of the black-fly season with longitude was found to be approximately at the rate of 1 day retardation for one degree of longitude eastward for the earliest emerging species, but to be almost negligible for species emerging late in the season.

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# Feeding and Reproduction of Black Flies (Simuliidae: Diptera)

By D. M. DAVIES  
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## ABSTRACT<sup>1</sup>

Nineteen Ontario species were studied and five from outside Ontario examined. Newly emerged females were of five types: with (1) mature eggs and reduced mouthparts (*Gymnopaia* spp., *Cnephia* spp.); (2) eggs half developed, much stored nutrient and reduced mouthparts (*C. emergens*, *P. alpestre*); (3) eggs half developed, much stored nutrient and piercing mouthparts (*C. mutata*); (4) eggs one-fifth to one-third developed, usually much stored nutrient, and piercing mouthparts (*Simulium* spp.); (5) eggs less than one-quarter developed, little stored nutrient and piercing mouthparts (*Prosimulium* spp., *Simulium* spp.). In *Cnephia* spp. (Type 1) mating occurred shortly after emergence with little flying. In *C. mutata* parthenogenesis is usual. Male mating flights occurred in species of *Prosimulium* and *Simulium*. But *S. decorum* mated on a support shortly after emergence and also just before oviposition. *C. mutata* fed infrequently on deer. Occasional females of *S. decorum* and *S. vittatum* (Type 4) fed on mammals. Species of type 5, *S. croxtoni*, *S. euryadminiculum*, *S. latipes* and *S. rugglesi* fed only on birds, *S. venustum* naturally on birds and mammals, and others naturally only on mammals. Ovarian development in species of types 4 and 5 took five or more days; in type 5 it began only after a blood meal that may take two weeks to acquire. Females of *Prosimulium*, *Cnephia*, and some *Simulium* species dropped eggs into the water while flying. Other *Simulium* species oviposited on water-covered surfaces either while flying or alighted. Oviposition was greatest in the evening but occurred at other times when populations were large and conditions favourable.

## DISCUSSION

T. H. G. AITKEN. I would like to know what techniques were used to determine what species of simuliids were feeding on the various hosts mentioned, e.g., bear, deer, beaver, otter, wild birds.

D. M. DAVIES. Part of this work was conducted at the Wildlife Research Station in Algonquin Park where animals were live-trapped. Also bears were shot and deer killed by cars on the highway. These animals were observed alive or freshly killed and the flies feeding on them could be observed and collected. Other flies fed when placed in vials inverted over the defeathered skin of birds. But most records were from birds and mammals on which flies were feeding before the approach of humans.

<sup>1</sup>For a complete account see *Can. Jour. Zool.* 34: 615-655. 1956.





# Ten Years of Blackfly Control in New York State

By D. L. COLLINS<sup>1</sup> AND H. JAMNBACK<sup>2</sup>

Albany, N.Y.

## ABSTRACT

Blackflies have long been a health problem and an economic handicap in northern New York State. With DDT and new methods of insecticide application, economically feasible control measures were developed. In 1945 and 1946, the use of fog generators against the adult flies was studied, and in 1948 the first large-scale regional control program was accomplished on 6000 acres in the Town of Webb. For this program Todd fogheads were used for the first time in a helicopter. Control measures were extended and modified by research until by 1956 about ten separate communities in northern New York with an aggregate of nearly 1000 square miles had some sort of blackfly control. Methods used include larviciding from airplanes, larviciding from the ground with DDT-impregnated plaster of paris blocks and other means, and control of adult flies by fogging and mist blowing. Each community pays for and executes its own program, usually on a township basis, with advice from the State Entomologist's office. Research established that the most important anthropophilic biting species in the region were *Prosimulium hirtipes* Fries, *Simulium venustum* Say and *S. tuberosum* Lund. It was found necessary to treat streams very early in spring to control overwintering larvae of *P. hirtipes*, but since *S. venustum* is in the egg stage at that time, a second treatment is made about three weeks later. DDT has been the best larvicide, and may be applied from airplanes at dosages as small as one-tenth of a pound per swath acre, at 1 gal. of 20% DDT solution per swath mile.

For many years the Adirondack Mountain region in New York State has been one of the most popular playgrounds and recreation areas in the United States. This same region also has a long history as a lumbering area and a source of other forest products such as pulpwood.

Throughout this area blackflies (Diptera: Simuliidae) are a serious economic handicap and health hazard. Of the 23 species of Simuliidae which have been found to occur in the region, only three are sufficiently annoying to humans to require control. These are *Prosimulium hirtipes* (Fries), *Simulium venustum* Say, and *S. tuberosum* Lund. Today, in 1956, about ten years after early tests first indicated that regional control of these blackflies might be feasible, the practical value and the popular appeal of control is such that there are about a thousand square miles in northern New York in ten separate localities, mostly in the Adirondack Park, where some sort of area blackfly control has been undertaken. These areas are shown on the accompanying maps (Figs. 1 and 2), with additional details in Table 1. The data in the table are further explained in the text.

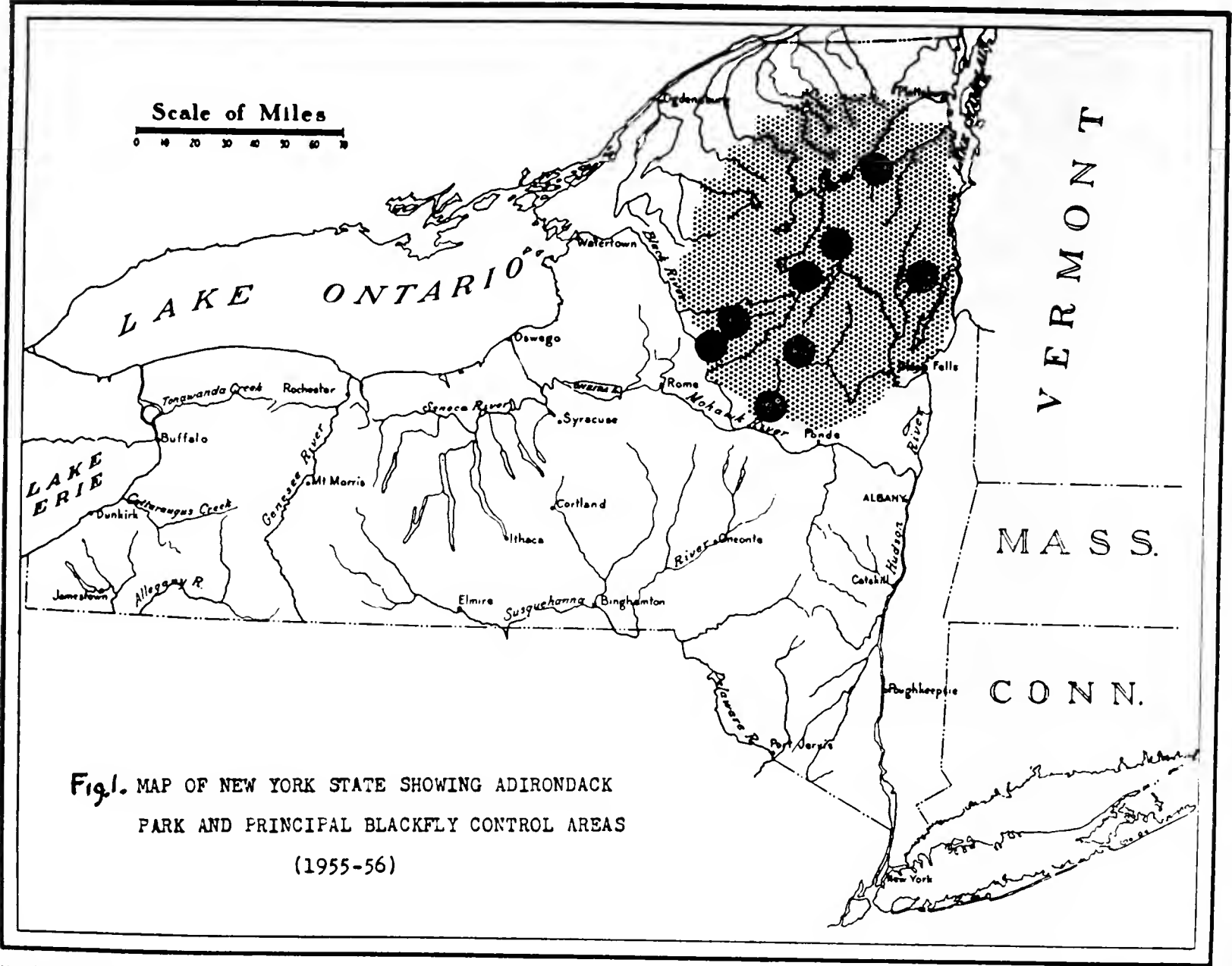
Much of Adirondack Park is uninhabited wilderness, and a large acreage, which belongs to the state, is set aside by a constitutional amendment to be kept "forever wild". These state lands, together with adjacent private property and surrounded villages, comprise some 5,600,000 acres (8,750 square miles) including all or parts of 11 counties and 82 townships. The total resident population of this area is around 30,000, an average of about 3½ persons per square mile.

The methods used in the control programs are not unique but in their application in the Adirondack resort areas there have been unusual features. Because of the low taxable population in the Adirondacks it might be expected that local groups could not or would not finance blackfly control programs. Yet, in all cases, these programs are financed locally or by large corporations having facilities in the area. The New York State Science Service provides aid only to the extent of working out the details of local control programs in cooperation with local groups.

The basic control methods were developed through research carried on by the New York State Science Service in cooperation with the Town of Webb, in northern Herkimer County, with headquarters in Old Forge. Here, in 1945, tests of one of the Todd smoke screen generators used by the Navy in World War II, adapted to generate insecticide fogs, were made by Dr. R. D. Glasgow. This "fog machine" was the ancestor of the modern

<sup>1</sup>State Entomologist, New York State Museum.

<sup>2</sup>Entomologist, State Science Service.



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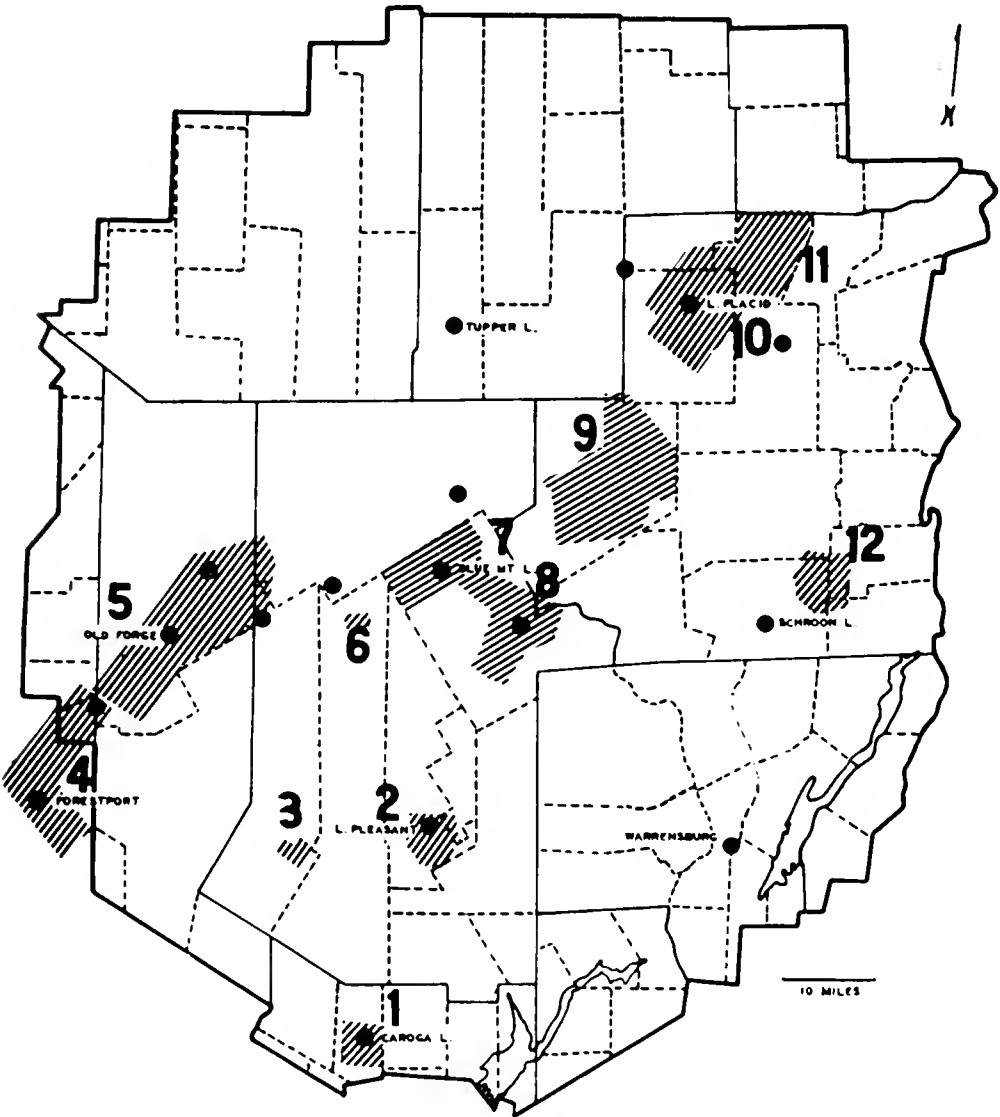


Fig.2. ADIRONDACK PARK  
BLACKFLY CONTROL AREAS (1955-56)

1. Caroga Lake - 25 sq. mi.
2. Speculator-Hamilton Lake - 50 sq. mi.
3. Star Sapphire Lake - 12 sq. mi.
4. Forestport - 100 sq. mi.
5. Town of Webb - 200 sq. mi.
6. Shedd Lake - 12 sq. mi.
- 7-8. Blue Mt. - Indian Lake - 175 sq. mi.
9. Newcomb-Tahawus - 124 sq. mi.
- 10-11. Lake Placid-Wilmington - 200 sq. mi.
12. Paradox Lake - 55 sq. mi.

Tifa, of which hundreds are now in use, in all parts of the world, for the control of blackflies, mosquitoes and other biting insects. Temporary adult blackfly control around a golf course was achieved, but it was not until 1948 that a large scale genuinely regional control

TABLE I. Outline of Blackfly Control Programs in New York State.

Reference number on fig. 2.	Name of area	Sponsoring agency	Approximate area included	Control measures used	Comments
	Caroga Lake	Town	25 sq. mi.	DDT plaster blocks and fogging	Fair control, blocks sometimes placed incorrectly
	Speculator-Hamilton Lake	Town and International Paper Co.	50 sq. mi.	Half by blocks, half by airplane larviciding	Better control reported in air-spray area
	Star Sapphire Lake	Onondaga Pottery Co.	12 sq. mi.	DDT-plaster blocks	Control data not available
	Forestport	Town	100 sq. mi.	In 1955, 2 aircraft larvicidings. In 1956, 1 air-spray and 1 blocking treatment	Control better in 1955. Early control good each year. 2nd treatment affected by flash floods in 1956.
	Town of Webb	Town	200 sq. mi.	Two aircraft larvicidings. Two blockings. Two foggings a day for a month	Generally good control throughout season. Some local annoyance from <i>S. venustum</i> at "difficult" sites
	Shedd Lake	Syracuse University	12 sq. mi.	Two aircraft larvicidings in 1955. Fogging only in 1956	Control fair to good in 1955, poor in 1956, prompted request for more air-spraying in '57
8	Blue Mt. Lake-Indian Lake	Town	175 sq. mi.	Two aircraft larvicidings plus some blocks	Good control of <i>P. hirtipes</i> . Control of <i>S. venustum</i> in 1956 poor for a week or more in mid-June, then better
	Newcomb-Tahawus	Town and National Lead Co.	124 sq. mi.	Two airplane larvicidings plus some blocks	Good control of <i>P. hirtipes</i> . Control of <i>S. venustum</i> in 1956 poor for a week or more in mid-June, then better.
11	Lake Placid-Wilmington	Towns	200 sq. mi.	Two aircraft larvicidings, partial plaster blocking, some fogging	Good control of <i>P. hirtipes</i> . Control of <i>S. venustum</i> in 1956 poor for a week or more in mid-June, then better
	Paradox Lake	N.Y. State Conservation Dept.	55 sq. mi.	Two and one-half aircraft larvicidings in 1955. No program in 1956	Good control

project was attempted. In that year a Bell helicopter, equipped with two Todd fog-heads attached to the ends of the exhaust stacks, was used for area fogging. With this apparatus, an average of about 500 acres of otherwise inaccessible terrain was fogged in a 3-hour flight day. About 6000 acres were fogged by the helicopter three times during the blackfly season of 1948. The fog oil solution was DDT dissolved in Sovacide F to make a 15 percent solution.

This helicopter fogging in the Town of Webb was the first regional blackfly control project in New York State. It proved to be so successful that blackfly control was thereafter accepted as a Town function, and funds have been appropriated by the Town every year since then for the program. However, methods used for blackfly control have undergone change through the years. Since regional fogging from a helicopter is costly, experiments were undertaken in the same area to test the feasibility of large-scale larviciding. Studies made by Dr. B. V. Travis and others in Alaska suggested that larviciding from aircraft might be practical in the Adirondack area. Since Dr. Travis had recently come to New York to assume duties as Professor of Medical Entomology at Cornell University, he was able to participate in and advise on the first airplane larviciding in the Town of Webb, in 1950.

During the same period, the use of DDT-impregnated plaster of paris blocks as described by Dr. Gustave Prévost, of Montreal, was tested as another method of larviciding. This method proved to have considerable popular appeal and is now one of the features of most of the control programs. Biological studies made by Dr. Gene deFoliart and Dr. Hugo Jamnback in the Old Forge area contributed to refinements and improvements in the control programs, and modifications were made from season to season as experience accumulated.

The biological, taxonomic and control studies on the Simuliidae of New York State, as outlined above, are presented in detail in two bulletins of the New York State Museum, namely, Bulletin 349, "The Black Flies of New York State," by Alan Stone and Hugo Jamnback, and Bulletin 350, "The Control of Blackflies in New York State," by Hugo Jamnback and D. L. Collins.

Several methods of blackfly control are used in the Adirondacks. They range from occasional, localized Tifa fogging to large-scale aircraft spraying or blocking programs, or to combinations of these methods. The basic method is larviciding, usually by aircraft; two complete coverages of a given control area are made, about four to five weeks apart. The first spray is applied in mid-April to eliminate the overwintering larvae of *P. hirtipes*, and the second is applied in mid- to late May to remove the larvae of *S. venustum* and *S. tuberosum*, which are in the less vulnerable egg stage at the time the spray for *P. hirtipes* is applied.

The airplane larviciding program is supplemented in most areas by the use of DDT-impregnated plaster of paris blocks. The blocks are tied in position in rapid portions of streams. The making and use of these blocks have been described by Prévost, Jamnback and Collins, and others. In addition to the larviciding, most of the programs include the occasional or regular use of Tifas (fog machines) to eliminate sporadic invasions of adult flies which either came in from outside the area, or came from larva which were not eliminated by the stream treatments.

To illustrate the points mentioned above in more specific terms the following details of the Town of Webb program as carried out in 1956 are cited:—

*Area covered:* Approximately 200 square miles.

*Methods used:* Larviciding by airplane spraying, supplemented by DDT-impregnated plaster of paris blocks, and adulticiding by Tifa fog machines.

*Equipment, materials, techniques of airspray:* Two Cessna 195 planes on floats, each discharging through two nozzles, one below each float. A 20% DDT solution was used, discharged at rate of 1 gallon per flight mile. The calculated dosage was about one-tenth pound of DDT per swath acre. The flight pattern consisted of parallel swaths one-quarter mile apart, with topical treatment of streams known to have been missed, and of "difficult" areas, as on mountain-sides. Sometimes extra passes were made over dams.

*Dates:* First air spray applied April 29—May 1. Second air spray applied June 4, 5 and 6. The applications were made later than usual in 1956 because of a rather cold spring.

*Total number of flight miles—*First and second spray each 990 miles.

*Airspeed while spraying:* 90 miles per hour.

*Gallons of spray used:* First and second spray each 990 gallons.

*Plaster blocks:* Placed at about time of sprays.

*Number of Plaster blocks used* (each containing 1 oz. of DDT): 1385 blocks.

*Labor of men placing blocks:* Approximately 50 man-days.

*Adulticiding:* 3 Tifas (1 in Eagle Bay, 1 in Big Moose, and 1 in Old Forge); were used regularly morning and evening for about a month, ending about July 10 whether needed or not. In Old Forge, used one 55-gallon drum of 20% DDT each morning and each night—a total of about 50 drums (2750 gallons) during the period of regular use.

The residents of the town are well satisfied with the program. It is now carried on every year as one of the Town services, like highway maintenance, snow removal and fire fighting.

From a research standpoint, with the described combination of several methods of larviciding and routine fogging to kill adults in one program, it is difficult now to determine which of the methods could be curtailed if necessary with the least harm to the control



effort. In the original control experiments made during the period 1948–1953, it appeared that either method of larviciding, i.e., by aircraft or by ground treatments such as DDT blocks, when carefully executed under favorable conditions could be expected to produce comparatively fly-free streams. It seemed further, that after a good larviciding program, fogging against the adults was not required except at “difficult” sites, and against occasional local and temporary invasions.

Where more or less complete control is demanded, rather than merely comparative relief, the tendency is to utilize both air and ground methods of larviciding on the theory that one method will “get” what the other misses. It is very important, in this connection, to note that the specific instructions for each larviciding method call for a minimum dosage which has been found by experiment to kill blackfly larvae without harming fish or other wildlife, and without seriously affecting most bottom-inhabiting stream insects. This dosage is so far below the level at which harm to most other stream inhabitants would result, that not only is each method, separately, considered safe, but also the combination of methods, would be expected to be safe. Any indication of serious harm to other stream fauna would be evidence that dosages in excess of those required for blackfly control had been employed.

In the Town of Webb, the site of the most extensive, most effective, and most complete blackfly control in the state, and where the methods described have been practiced over the longest period, stream fishing, in 1956, was reported as being the best in several years.

It has long been felt that the size of the control area is an important factor in the success of the control measures used. Thus, protection in the center of a 200-square-mile area such as the Town of Webb might be expected to be better than that afforded in a smaller area. To study the effectiveness of control in a small area, a 50-square-mile plot around Paradox Lake was laid out, in 1955. The blackfly control program consisted of two complete larval airplane sprays, one for *P. hirtipes* and one for *S. venustum* and *S. tuberosum*, with a partial coverage between the regular spray dates, applied to warmer streams, in which blackfly development proceeded more rapidly.

Statistics for the Paradox Lake program were as follows:—

*Equipment used:* Stearman plane equipped with 30-foot boom and using 2 nozzles.

*Area covered:* A square plot 7.5 miles to 8 miles on a side, comprising about 37,000 acres.

*Technique:* A regular swath pattern with parallel swaths 1/4 mile apart, with topical touch-up applications where needed in difficult spots.

*Material:* Approximately 20 percent DDT solution; i.e., 2 pounds of DDT in each gallon of solvent.

*Solvent:* Sovacide F.

*Dosage:* One gallon of solution per flight mile.

*Number of sprays:* Two complete coverages, the first on April 20 for the early spring species (*P. hirtipes*), and the second on June 16 for the later species (*S. venustum*), with a partial, topical coverage of warmer streams on May 16 to kill early-developing *S. venustum*.

*Other information:* Each complete coverage required 4½ to 6½ hours of flying time.

No measures other than the larvicide air-spraying were used at Paradox. The results achieved were comparable to those obtained where several methods were used, thus indicating that under favorable conditions in a “good” site, in a normal year, airplane larviciding alone may be all that is needed. However, conditions are seldom sufficiently favorable to obtain complete success with one method alone. Control of *P. hirtipes*, which overwinters in the larval stage, is almost always very good, whether larvicide is applied by aircraft or by blocks. The larvicide is applied when there is no foliage on the deciduous trees and when the spring snow melt run-off makes the streams full and swift. These factors appear to be favorable for good control.

Control of *S. venustum* and *S. tuberosum* by application of larvicide from aircraft has been less uniformly satisfactory. These species apparently overwinter in the egg stage, appearing as larvae in the late spring. The exact time of appearance depends on the temper-

ature of the individual streams. In the Adirondacks there is no one time when all of the streams can be treated to destroy *S. venustum* and *S. tuberosum* larvae. By the time larvae appear in some of the colder, spring-fed streams, larvae from the warmer pond outlets, beaver dam streams and "flows" have pupated and, in many cases, are on the wing as adults. Further complications developing at this time include slower stream flow and the appearance of heavy foliage, making control by aircraft spraying less reliable, particularly in the smaller streams.

All these difficulties were particularly troublesome in 1956, when the optimum time for the *S. venustum* sprays varied from stream to stream even more than usual because of the long cold spring. The effect of these factors was reflected in a period of *S. venustum* annoyance in most control areas in mid-June. The possibility that the use of granular or pelletized formulations might overcome some of these difficulties in the second spray is now receiving study.

#### DISCUSSION

J. FRAGA DE AZEVEDO. Can you localize your treatments or must all parts of all streams be treated?

D. L. COLLINS. In our area breeding may occur in all sections of suitable streams. In airplane larviciding we cannot be selective. Therefore, a regular swath pattern, with swaths 1/4-mile apart, is used when topography permits.

W. E. SNOW. Can you estimate the height of flight and the amount of insecticide reaching the stream surface through vegetation?

D. L. COLLINS. We have no accurate estimate of the amount of insecticide actually reaching the stream. We check by observing oil droplets on quiet stretches of water. The height of flight varies according to topography; 50 to 100 feet above tree-tops might be an average.

# Black Flies (Diptera: Simuliidae) of the Agricultural Areas of Manitoba, Saskatchewan, and Alberta<sup>1</sup>

By F. J. H. FREDEEN  
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## ABSTRACT

Twenty-five species of black flies were collected from an area in the three provinces lying between the 49th and 55th parallels of latitude in the period 1947 to 1955 inclusive. Two additional species were previously reported. The immature stages are restricted to flowing water, and a complex of species was found in most of the 801 collections from the 283 streams and rivers examined. The females of many species require a blood meal for the maturation of eggs. Productivity of animals and man is affected by attacks by *Simulium arcticum*, *S. venustum*, *S. vittatum*, *S. meridionale*, and *S. luggeri*. A single outbreak of *S. arcticum* was responsible for more than 600 domestic livestock fatalities. Swarms of *S. arcticum* have been carried more than 140 miles by the wind. The flight range of other blood-sucking species was found to vary from 10 miles (*S. venustum*) to 37 miles (*S. vittatum*). Some species overwinter in the egg stage and others as larvae. Annual control of *S. arcticum* and *S. venustum* has been achieved in rivers with volume flows of up to 65,000 cubic feet per second by eliminating larvae with single 15-minute, 0.1 p.p.m. applications of DDT. A single application was effective as far as 115 miles downstream. Larvae were killed by ingesting silt particles with adsorbed DDT.

## INTRODUCTION

The Simuliidae (locally known as black flies, "sand flies", or buffalo gnats) of the western Canadian prairie regions were the subject of special investigations by the Entomology Division, Canada Department of Agriculture, in the years 1947 to 1955 inclusive. The systematics aspect of the study included 801 collections from 283 streams and rivers from the agricultural areas of Alberta, Saskatchewan, and Manitoba, an area bounded on the north by approximately the 55th parallel of latitude and on the south by the 49th. In addition the life-cycles, economic importance, and control of the more abundant species were studied. This is a report of some of the major findings.

## THE SPECIES PRESENT

Table I is a list of the species of black flies as represented by the immature stages collected from streams and rivers in each of the three provinces. Twenty-five species, of which three are yet unnamed, were found in the area. Two additional species, *Prosimulium fulvum* (Coq.) and *Simulium pictipes* Hagen were reported from the area by Strickland (1938).

*Simulium vittatum* Zett. and *S. venustum* Say were each found in about 40 per cent of the collections, and *S. arcticum* Mall. and *S. luggeri* N. and M. were also relatively abundant. The adults of these and of *S. meridionale* Riley, *S. griseum* Coq., and *S. rugglesi* N. and M., are the blood-sucking species which are sometimes sufficiently abundant to reduce the production of domestic livestock and poultry or to hamper the outdoor activities of man in this region. Outbreaks of one or more of these seven species occur annually in some localities, and livestock fatalities sometimes result from outbreaks of *S. arcticum*.

## SIMULIUM ARCTICUM

*S. arcticum* is the most important black fly in the area in terms of measurable losses. It is primarily a pest of animals and rarely bites man. In Saskatchewan livestock have been killed by outbreaks of this species since the days of earliest settlement, and since 1944 there have been outbreaks almost every year with more than 1,100 fatalities in livestock. On June 2 and 3, 1946, a single outbreak was reported to have killed more than 600 animals (Rempel and Arnason, 1947). All of these outbreaks occurred in central Saskatchewan only, and in an area adjacent to the South Saskatchewan River, although *S. arcticum* is widespread elsewhere in Western North America.

<sup>1</sup>Contribution No. 3564, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

TABLE I. List of 25 Black Flies Collected from the Agricultural Areas of Manitoba, Saskatchewan, and Alberta, 1947 to 1955 Inclusive.

	Manitoba	Saskatchewan	Alberta	Notes
<i>Cnephia dacotensis</i> (D. & S.)	+	+	0	rare
<i>C. taeniatifrons</i> End.	0	+	0	rare
<i>Cnephia</i> "N"	0	+	0	rare
<i>C. saileri</i> Stone	0	0	+	very rare
<i>Simulium aureum</i> Fries	+	+	+	widely distributed
<i>S. canoniculum</i> (D. & S.)	0	+	0	rare
<i>S. latipes</i> (Meigen)	+	+	0	rare
<i>S. pugetense</i> (D. & S.)	0	+	+	rare
<i>Simulium</i> "2"	0	+	0	rare
<i>S. vittatum</i> Zett.	+	+	+	widely abundant
<i>S. arcticum</i> Mall.	0	+	+	locally abundant
<i>S. bivittatum</i> Mall.	0	0	+	rare
<i>S. corbis</i> Twinn	0	0	+	very rare
<i>Simulium</i> "C"	0	0	+	rare
<i>S. decorum</i> Walker	+	+	+	widely distributed
<i>S. griseum</i> Coq.	0	+	+	rare
<i>S. hunteri</i> Mall.	0	+	0	rare
<i>S. luggeri</i> N. & M.	+	+	+	widely distributed
<i>S. malyshevi</i> D., R. and V.	0	0	+	very rare
<i>S. meridionale</i> Riley	+	+	+	locally abundant
<i>S. rugglesi</i> N. & M.	+	+	0	locally abundant
<i>S. sayi</i> D. & S.	0	+	+	rare
<i>S. transiens</i> Rubtzov	0	+	+	rare
<i>S. tuberosum</i> (Lund.)	+	+	+	widely distributed
<i>S. venustum</i> Say	+	+	+	widely abundant

The life-history of *S. arcticum* was studied in detail. It overwinters as eggs in the sand of the river bed (Fredeen *et al.*, 1951). The eggs are deposited singly by females flying over the surface of the river, instead of in masses on objects at the water margin like those of many other species. The eggs settle to the bottom where they remain until hatching commences after the break-up of ice the following spring. As many as 570 eggs were extracted from one cubic foot of sand dredged from the river bed. The newly-hatched larvae attach to pebbles in the shallow rapids, but the larger larvae are found massed on boulders in the swiftest flowing water of the major rapids. Populations as dense as 450 larvae and pupae per square inch have been counted, and based on such counts it was once estimated that there were more than seven billion pupae in a single rock-filled weir across the North Saskatchewan River at Prince Albert. This was on June 9 and 10, 1947, just prior to a damaging outbreak.

The larvae are indiscriminate feeders. Particles in the gut contents of dissected larvae were almost 100 per cent inorganic with particle sizes ranging up to 0.06 mm. in diameter. The success of black-fly larvicide in the Saskatchewan River is apparently the result of larvae ingesting suspended particles containing adsorbed DDT (Fredeen *et al.*, 1953).

The average date of appearance of pupae in the river is May 28, and this stage lasts for five to seven days. The average date of beginning of spring outbreaks of adults is June 5. Males apparently commence to emerge a day or so ahead of the females, and mating occurs in swarms at distances of up to 40 miles from the river just prior to blood-feeding. Only the females take a blood meal, and swarms of these are carried by the wind for great distances. Livestock were killed on one occasion more than 140 miles from the point of origin of the flight.

The exact mechanism of damage is not yet known. Disease organisms, suffocation by inhaling flies, anaphylactic shock, and direct toxemia have been suggested. Post mortems (Millar and Rempel, 1944) failed to reveal disease organisms, but the finding of oedema of the lungs and large quantities of serum in the body cavities suggested that the deaths were the result of shock induced by toxemia.

Tests to control *S. arcticum* in the Saskatchewan River, which has a volume flow of up to 65,000 cubic feet per second, were begun in 1948. These showed that infestations of larvae could be eliminated for distances of up to 115 miles in the river by a single 15-minute application of 0.1 p.p.m. of DDT applied as a 10 per cent solution of DDT in



kerosene or similar solvent (Fredeen *et al.*, 1953). This larvicide was selective in action in that blackfly larvae which ingested silt particles with adsorbed DDT were killed, whereas other aquatic larvae that did not regularly feed on suspended silt particles were affected to a lesser degree, and fish were not harmed.

The annual control program includes the assessment of populations of larvae in the larger rapids during the first two weeks of May and five to seven weeks after the ice-break-up in the spring. The locations and timing of the applications of larvicide are determined by the stage of development and density of infestations. Applications, when required, are made just before the beginning of pupation. The results of each application are checked three to five days later.

In the nine years since 1948, 10 outbreaks of *S. arcticum* occurred, but these resulted in the loss of only 29 animals as compared with more than 1,100 animals in the five outbreaks immediately preceding the beginning of control in 1948.

Since minor outbreaks still occur, despite the annual control program, livestock owners within 100 miles or more of the major breeding places are warned through the radio and the press of the possibility of outbreaks just before the beginning of adult emergence each year. They are warned that black flies may appear suddenly on any day when the wind is blowing from the river, and at the first sign of black flies to confine bulls and valuable dairy cattle to barns. Smoke from smudges is commonly used to protect range cattle, and mineral oil swabbed along an animal's underline where these black flies generally concentrate their attacks is an effective repellent. Commercial livestock insecticides are not widely used, largely because of a lack of experimental evidence as to their effectiveness in preventing black-fly damage.

#### SIMULIUM VENUSTUM

Second in economic importance to *S. arcticum* in this region is *S. venustum*. Unlike *S. arcticum* it is widespread in Canada and is a pest of man as well as of animals. Furthermore, it is rare in large rivers but abundant in many streams and small rivers. It has a relatively short flight range with a maximum known range of about 10 miles as compared to 140 for *S. arcticum*. Because of this short flight range, outbreaks of *S. venustum* only occur in the vicinity of its main breeding places.

*S. venustum* has a life-history which resembles *S. arcticum* in some respects. It overwinters in this region as eggs in the sand of stream beds, and hatching commences one or more weeks after the ice breaks up in the spring. Adults commence to emerge five to eight weeks after the break-up.

There are several generations of *S. venustum* in a summer, but generally the adults of only the first generation are sufficiently abundant to cause concern, and populations decline with each succeeding generation. Stone and Jamnback (1955) consider that *S. venustum* consists of a complex of species and suggest that the true *S. venustum* has only one generation per year. However, the species which we consider to be *S. venustum* lays eggs which commence to hatch within about four days and produce another generation of adults in less than a month.

We were successful in rearing *S. venustum* in the laboratory, and under optimum conditions adults commenced to emerge 13 days after the introduction of eggs. Two peculiarities which were observed included a great variation in the rate of growth of individual larvae under apparently identical conditions, and a sex ratio of one male to two females. Davies (1950) reported a ratio of 1:1.5 from emergence cages in the field.

Outbreaks of *S. venustum* severe enough to reduce milk production in dairy cattle by about 50 per cent and to prevent weight gains in beef animals have been reported almost every year. These outbreaks also sometimes prevent man from carrying out his normal outdoor activities. Fortunately these outbreaks of *S. venustum* last only one to two weeks.

Outbreaks do not occur every year from any one stream, but are apparently induced in many instances by high river levels in the spring. Thus when there is a rapid flow of water through grass and willows on flooded river bench lands for three or four weeks, the development of unusual numbers of larvae is encouraged. Relatively few are produced when a river remains within its banks in the spring except where there are natural obstructions such as rock-filled rapids in which the larvae attach.

*S. venustum* is generally considered to be the chief vector of *Leucocytozoon simondi* M. & L. a blood protozoon of birds (O'Roke, 1934). Savage and Isa (1945) reported an outbreak of *Leucocytozoon* in turkeys in Manitoba near the Assiniboine River that killed about 5,000 out of a flock of 8,000, but the black-fly vector was not determined. Burgess (in press) reported that *Leucocytozoon simondi* M. & L. was rare in Saskatchewan and Manitoba, and that it was recovered from three species of wild ducks.

Shewell (1955) indicates that *S. rugglesi* may be responsible for transmitting *Leucocytozoon simondi* to ducklings and goslings, and he suggests that the specimens that O'Roke worked with may have been *S. rugglesi* rather than *S. venustum*. Further work is required to determine the part that *S. rugglesi* and *S. venustum* play in the natural transmission of *Leucocytozoon*.

Black-fly larvicide tests were conducted on the Souris River in 1951 and 1953 and on the Beaver River in 1956 in attempts to prevent outbreaks of *S. venustum*. The larvicide used was a 10 per cent solution of DDT in kerosene. The applications consisted of 0.1 to 0.12 p.p.m. of DDT for 15 minutes. Single applications on the Souris River were found to be almost 100 per cent effective for about 13 miles downstream but not more than 50 per cent effective at a distance of about 30 miles. On the Beaver River a single application was almost completely effective in eliminating larvae four and 12 miles downstream, the only points investigated.

#### SIMULIUM VITTATUM

A third species investigated, *S. vittatum*, is the most widespread species of black fly in the region and the immature stages were found in a wider variety of stream conditions than were those of any other species. The typical habitat was a stream draining a warm lake or swamp and containing water rich in suspended algae.

Unlike the other common species of black flies, *S. vittatum* overwinters as larvae instead of eggs in water flowing under the ice in small permanent streams. Temporary streams in the area are repopulated each summer from eggs laid by adults flying in from permanent streams. There are several generations in a year, and population density increases with each succeeding generation during the warm part of the summer and at a time when other species are becoming less abundant. The adults of *S. vittatum* are the last to disappear in the fall and are sometimes observed attacking livestock when the streams are partly frozen over. Under natural conditions the life-cycle during the summer is completed in less than a month. In the laboratory, adults were produced from eggs in as short a time as 14 days.

*S. vittatum* attacks a wide variety of domestic animals and outbreaks are sometimes annoying to livestock and man. However, it is not as important a pest as either *S. arcticum* or *S. venustum*, and two factors in particular are responsible for this. Firstly it develops in abundance only in the smaller streams, and secondly the females have a flight range of at least 37 miles and do not always attack in swarms where they emerge but are scattered across the countryside.

In control tests, larvae of *S. vittatum* were as readily eliminated with small amounts of DDT as were those of *S. arcticum* and *S. venustum*.

#### OTHER PEST SPECIES

##### SIMULIUM MERIDIONALE

This species is widely distributed and occasionally abundant in the southern agricultural areas of the three provinces. However, there are only one or two reports of its attacks affecting the productivity of poultry or livestock. Blood-sucking females have been collected as far as 18 miles from the nearest point of emergence. Field collections indicate more than one generation in a summer.

##### SIMULIUM LUGGERI

The immature stages of *S. luggeri* occur mainly in small rivers in the northern half of this region. It is a multiple generation species with adults abundant from mid-June to late July. The adults attack cattle and horses as far as 15 miles from the streams from which they emerge, but definite losses cannot be attributed to this species.

## SIMULIUM RUGGLESII

In this region the immature stages of *S. rugglesi* are found in only a few small rivers but adults sometimes emerge in abundance from these during the first two weeks of June. Blood-sucking is apparently limited to avian hosts, and Shewell (1955) indicates that *S. rugglesi* may be responsible for transmitting *Leucocytozoon simondi* M. & L. to ducklings and goslings in Eastern Canada.

## SIMULIUM GRISEUM

*S. griseum* occurs mainly in the Milk and Saskatchewan rivers of southern Alberta. In July, 1944, it severely attacked horses at Medicine Hat, Alberta, but elsewhere in this region it has not proved to be a pest species.

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# Phlébotomes et Leishmaniasés Autochtones en France<sup>1</sup>

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## RÉSUMÉ

Les craintes exprimées par divers auteurs (l. Nattan-Larrier et L. Grimard, 1935; J. Guilhaon, 1950) concernant l'extension possible des leishmaniasés autochtones canines ou humaines méditerranéennes à la Région parisienne, au Centre et à l'Ouest de la France, semblent, à première vue, justifiées par la répartition mieux connue des phlébotomes vecteurs, *Phlebotomus perniciosus* Newstead et *P. papatasi* Scopoli.

L'étude de toutes les récoltes, anciennes ou nouvelles, de ces Diptères et aussi des caractéristiques météorologiques des localités où elles furent signalées, confirme que, dans ces régions extra-méditerranéennes, il ne peut exister qu'une génération annuelle de ces insectes et la courte durée où l'on observe les adultes piqueurs y rend difficile, sinon improbable, la transmission des leishmaniasés; ainsi s'explique donc la rareté des cas observés dans ces zones plus septentrionales, cas aberrants dont la transmission, pour certains, serait peut être assurée différemment.

En France, depuis les premières observations de leishmaniasé viscérale canine autochtone (Pringault, 1914) à Marseille, ou humaine (M. Labbé, Targhetta, Ameuille, 1918) à Nice, de nombreux auteurs ont montré l'importance de ce foyer méditerranéen qui s'étend à l'intérieur même du pays. La leishmaniasé viscérale humaine autochtone, en effet, a été signalée non seulement dans les départements méditerranéens de Corse, Alpes maritimes, Bouches-du-Rhône, Var, Hérault (vraisemblablement Aude et Pyrénées orientales), mais encore dans ceux des régions limitrophes (Vaucluse, Gard, Lozère, Ardèche, Isère et Rhône), de l'Est (Vosges). La leishmaniasé canine existe en Corse, Alpes-maritimes, Bouches-du-Rhône, Var, Haute-Garonne, Pyrénées-orientales, Basses-Pyrénées(?), Rhône, Allier, Loire-Inférieure, Seine, Seine & Oise(?). L'existence d'une leishmaniasé cutanée autochtone ou Bouton d'Orient n'a été reconnue que plus récemment depuis les premières observations dans les Pyrénées-Orientales (Ravaut, 1920) et dans la région de Nice (J. Nicolas, B. Spinetta et G. Massia, 1943). Elle n'existe que dans quelques départements méditerranéens ou voisins (Alpes maritimes, Hérault, Gard, Pyrénées-Orientales).

La répartition des stations reconnues de *P. perniciosus* vecteur de *Leishmania donovani* infantum, d'après la dernière revue critique (J. Raynal, 1954) complétée de diverses observations (C. Toumanoff et R. Chassignet, 1954; M. Sicart, 1954) et de récentes déterminations (J. Colas Belcour et J. Rageau, 1956) se limite aux départements suivants: Corse, Alpes-maritimes, Var, Bouches-du-Rhône, Pyrénées-orientales, Gard, Ardèche, Haute-Garonne, Lot, Dordogne, Gironde, Charente, Charente-maritime, Puy-de-Dôme, Allier, Vienne, Indre et Loire, Saône et Loire, Rhône, Côte-d'Or, Maine et Loire, Mayenne, Eure et Loir, Seine, Seine et Oise, Seine et Marne; celle de *P. papatasi*, vecteur de la leishmaniasé cutanée à *L. tropica* comprend: Corse, Var, Bouches-du-Rhône, Vaucluse, Hérault, Gard.

L'altitude des gîtes de *P. perniciosus* est fonction de leur latitude. La plus élevée observée sur le littoral méditerranéen atteint 900 m. à Azzana (Corse)<sup>2</sup> ou 730 m. à St. Vallier du Thiey (Alpes maritimes), ne dépasse pas 322 m. dans le Centre (Clermont Ferrand, Puy-de-Dôme), 190 m. dans le Sud-Ouest (Charras, Charente), 316 m. dans l'Est (Dijon, Côte-d'Or) et 146 m. dans la région parisienne (Etampes, Seine & Oise). L'altitude des lieux où furent observés des cas de leishmaniasé viscérale coïncide avec celle des gîtes de *P. perniciosus*.

Les rares *P. papatasi* récoltés furent capturés de 0 à 400 m.

La saison des adultes de *P. perniciosus* d'après les auteurs varie également suivant la situation géographique des lieux de leurs observations, depuis la Corse où elle s'étend de la fin-mai jusqu'à mi-octobre, le littoral méditerranéen de la mi-juillet à la mi-octobre, jusqu'au Centre, l'Ouest ou la région parisienne, où elle ne dépasse guère juillet et août.

<sup>1</sup>Nous ne donnons ici qu'un résumé de notre Mémoire qui, dépassant la limite fixée par ces Proceedings, paraîtra prochainement in extenso avec ses références bibliographiques dans les Archives de l'Institut Pasteur du Maroc, t.v. cahier VI, 1957, pp. 243-61.

<sup>2</sup>Encore s'agit-il pour cette localité (citée par R. M. Nicoli, 1952) de la variété insulaire, *P. perniciosus legeri*, (J. Mansion, 1913).

D'une étude d'ensemble des durées des cycles évolutifs expérimentaux de *P. perniciosus* rapportés par divers auteurs, des températures moyennes et minima moyennes de tous les lieux de capture ou des stations météorologiques voisines à la saison des phlébotomes adultes (d'après le recueil des données statistiques relatives à la climatologie de la France, J. Sanson, 1953), on peut encore tirer plusieurs conclusions. Il n'y aurait qu'en Corse (température moyenne 22°9C) et peut-être en quelques départements côtiers méditerranéens ou limitrophes (t. m. de 20°6 à 21°2 C) dans les années les plus chaudes, qu'il existerait

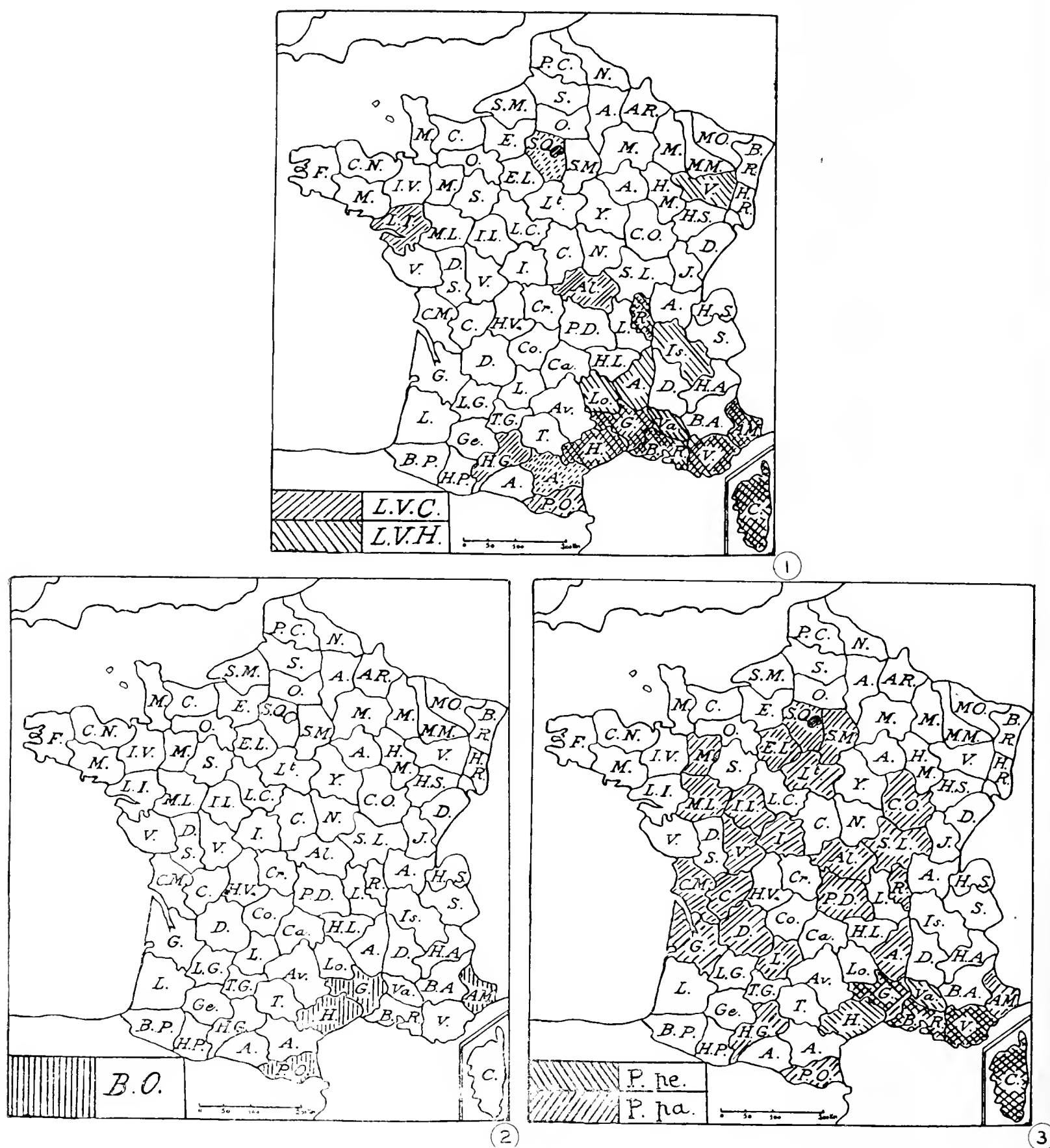


Fig. 1. Répartition de la leishmaniose viscérale canine (L.V.C.). Répartition de la leishmaniose viscérale humaine (L.V.H.). Fig. 2. Répartition de la leishmaniose cutanée ou Bouton d'Orient (B.O.). Fig. 3. Répartition du *Phlebotomus perniciosus* (P. pe.). Répartition du *Phlebotomus papatasi* (P. pa.).

deux générations annuelles, l'une l'hiver née des larves des pontes de l'année antérieure, l'autre d'été, la plus importante et donc la plus dangereuse, éclore des larves provenant des pontes de la génération précédente confirmation théorique des observations faites en Corse (J. Mansion, 1914, M. Nicoli, 1951). Par contre, en les seuls mois de juillet-août dans les départements du centre (t.m. 18°9 à 21° C) de l'Ouest (t. m. 18°1 à 19°2 C) dans la région parisienne (t. m. 18°3 à 18°8 C) une seule génération est possible; ces données confirmeraient ainsi notre opinion émise précédemment (J. Colas Belcour et J. Tisseuil, 1936) R. Durand-Delacre, 1949). Un échelonnement des éclosions des imagos expliquerait la durée de leur

saison; l'hibernation de ses larves en leur quatrième stade peut être facilitée par l'allongement de sa durée sous l'influence du froid et de la diapause: la faible densité phlébotomienne due probablement à la mortalité post-hibernale parfois constatée et à la courte longévité des adultes, est cause de la rareté des cas de leishmaniose viscérale observée dans les régions extra-méditerranéennes.

La non-concordance dans l'aire géographique de l'endémie de la leishmaniose viscérale humaine ou canine et celle des *P. perniciosus* s'explique par la répartition des entomologistes spécialistes qui commande souvent leurs prospections et les difficultés bien connues, inhérentes à la recherche de ces Diptères (influence défavorable des pluies d'été, du vent, de l'abaissement de la température et de la pression barométrique, brièveté de leur vie adulte et cycle annuel unique peu important dans les régions les plus septentrionales). Parfois, certaines leishmanioses viscérales de localités plus éloignées des régions méditerranéennes (notamment le cas humain signalé dans les Vosges par Pehu et Bertoye, 1937) pourraient s'expliquer, soit par la transmission mécanique due à un arthropode (Stomoxe ou Rhipicephale), soit même par une contamination directe de l'enfant qui, en caressant ou se faisant lécher par les chiens du voisinage, infestés de leishmaniose au cours d'un séjour plus méridional, s'infecte lui-même avec les leishmanies éliminées dans les sérosités des ulcères de la peau ou des muqueuses; cette hypothèse trouve sa confirmation dans une contamination accidentelle de laboratoire récente (L. L. Terry, J. L. Lewis & S. M. Sessoms, 1950) qui ne permet plus d'en exclure la possibilité.

Les caractéristiques altimétriques et climatiques des localités, où ont été observés les cas de bouton d'Orient véritablement autochtones, sont peu précises vu le faible nombre des observations. Le vecteur semble être exclusivement *P. papatasi* dont la répartition coïncide avec cette dermatose en trois départements. Le cycle évolutif de *P. papatasi*, un peu plus long que celui de *P. perniciosus*, nous fait croire qu'il n'a, lui aussi, sauf dans des années particulièrement chaudes, qu'une seule génération annuelle.

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### DISCUSSION

J. FRAGA DE AZEVEDO. Je voulais demander au Dr. Colas-Belcour comment est-ce qu'a été vérifié la mort de larves de *Phlebotomus*, au nord de la France, au commencement du temps froid.

J. COLAS-BELCOUR. La mortalité de la première génération du début de l'été serait d'après les auteurs palestiniens la plus importante, et expliquerait le faible nombre de *Phlebotomus*. La seconde, plus nombreuse, est réputée la plus dangereuse. Cette mortalité ferait suite à la période post-hivernale pour la première génération.



# La Présence de l'Espèce *Phlebotomus perfiliewi* dans la République Populaire Roumaine Oecologie et Phénologie<sup>1</sup>

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## RÉSUMÉ

Les espèces de phlébotomes identifiées jusqu'en 1955 sont: le *Ph. papatasii* Scop. et le *Ph. longiductus*. En 1955, des investigations entomologiques déterminées par l'apparition pendant l'année précédente de quelques cas de leishmaniose viscérale dans une région située au sud-ouest du pays (région de Craiova) ont conduit à l'identification de l'espèce *Ph. perfiliewi* (Parrot 1930; sin. *Ph. macedonicus*, Adler et Theodor 1931) dans la région respective. Cette espèce a été trouvée dans trois métairies situées à la périphérie de deux localités, sur les six contrôlées. La densité était très réduite (exemplaires sporadiques) dans les maisons et les dépendances ainsi qu'à l'extérieur. (Dans deux des maisons au *Ph. perfiliewi* on avait enregistré des cas de leishmaniose viscérale). Les conditions climatologiques de 1955 n'étant pas favorables au développement des phlébotomes.

Néanmoins, les investigations effectuées dans des biotypes naturels (terriers) de *Citellus citellus* et nids de *Merops*, ont démontré la présence de nombreux exemplaires de *Ph. perfiliewi*. La grande fréquence du *Ph. perfiliewi* dans les biotypes naturels et sa présence sporadique dans les collectivités humaines, plaiderait pour le caractère sauvage de cette espèce dans notre pays—bien qu'il ne soit pas exclu que pendant les années caractérisées par des conditions climatologiques favorables, la hausse encore plus grande (accentuée) de la densité dans les biotypes naturels ne détermine une migration plus accentuée vers les collectivités humaines.

## INTRODUCTION

Pendant l'année 1954, on a signalé dans une région du sud-ouest du pays (région de Craiova) l'apparition d'un foyer de leishmaniose viscérale (Minculescu, et al., 1955).

Etant donné que la leishmaniose viscérale était presque inconnue sur le territoire de notre pays (les premiers et les derniers deux cas étant communiqués en 1919 par le Prof. Manicatide parmi les problèmes à étudier qui se posaient en relation avec cette affection, se trouvait aussi celui de l'identification de l'insecte vecteur, respectivement de l'espèce de phlébotome transmetteur.

Jusqu'en 1955, l'année où nos recherches et nos investigations ont commencé, on ne connaissait dans notre pays que deux espèces de phlébotomes: *Ph. longiductus*, mentionné seulement dans deux localités (Nitzulescu et Nitzulescu 1928), et *Ph. papatasii* à vaste aire de distribution au sud et à l'ouest du pays, zones où il a déterminé—à partir de l'année 1939—des épidémies intenses de fièvre à pappataci (Leon 1910, Duport et Teodorescu 1946, Ionescu-Mihăilesti, et al., 1940, Zaharia et Zaharia, 1946, Lupascu, et al., 1956).

Bien que l'aire de propagation où l'on a signalé le foyer de 1954 coïncide avec l'une des zones de répartition de l'espèce *Ph. papatasii*, toutefois, en tenant compte du fait que la plupart des auteurs accordent au *Ph. papatasii* un rôle réduit ou même nul dans la transmission de la leishmaniose viscérale, nous nous sommes proposé comme première étape, d'entreprendre les recherches les plus complètes sur la faune phlébotominae de la zone respective et d'établir si, outre les *Ph. papatasii*, l'on ne trouve pas aussi d'autres espèces de phlébotomes<sup>4</sup>.

Dans ce but, en tenant compte des données connues dans la littérature universelle ainsi que des méthodes employées et du succès des investigations des chercheurs soviétiques (Petrisceva), nous avons entrepris d'étendre nos recherches aussi à certains biotypes naturels, situés à de différentes distances en dehors des villages, c'est-à-dire: terriers de rongeurs, reptiles, nids d'oiseaux etc.

Ces recherches, commencées au courant de l'année 1955, ont eu pour résultat l'identification de l'espèce *Ph. perfiliewi* (Parrot 1930), synonyme du *Ph. macedonicus* (Adler et Theodor 1931, Nitzulescu 1933) non-signalée jusqu'à présent dans notre pays.

<sup>1</sup>Étude effectuée en collaboration avec le Sanepid de Craiova et les stations de Malaria Corabia et Calafat.

<sup>2</sup>Membre correspondant de l'Académie de la République Populaire Roumaine.

<sup>3</sup>L'Institut de Microbiologie, Parasitologie et d'Epidémiologie "Dr. I. Cantacuzino".

<sup>4</sup>D'après les données disponibles de la littérature universelle, seulement en Espagne le *Ph. papatasii* est considéré comme le vecteur principal de la leishmaniose viscérale (Craig et Faust 1949, Mackie, et al., 1949). En outre, quelques auteurs soviétiques aussi (Gromasevski et Waindrah 1947, Ivassentov, et al., 1951) l'accuse d'être parmi les transmetteurs de la leishmaniose viscérale dans les républiques de l'Asie Centrale.

Dans l'étude entreprise, les observations entomologiques ont eu pour objectif:

- 1) *L'investigation des densités phlébotomiennes.*
  - a) dans les habitations (chambre à coucher, autres chambres etc).
  - b) dans les biotopes naturels (tanières d'animaux sauvages, de rongeurs, de reptiles et dans des nids d'oiseaux);
- 2) *L'identification des espèces capturées.*
- 3) *L'enregistrement des observations concernant la biologie, l'oecologie et la phénologie des espèces présentes.*

### MÉTHODES DE TRAVAIL

Les densités phlébotomiennes ont été appréciées: (a) dans les collectivités, en enregistrant le nombre des exemplaires adultes (♂-♀) dans 5 à 15 foyers contrôlés dans chaque localité; (b) dans les biotopes naturels, en enregistrant le nombre d'exemplaires capturés sur du papier collant ou dans un "entonnoir recouvert d'une éprouvette".

Le papier collant a été préparé de 1 à 24 heures avant l'usage, en papier vélin ou semi-vélin (20/15 cm.), enduit d'une mince couche d'un mélange d'huile de ricin—colophonium en proportion de 4-5%, préparé au chaud. Le papier collant ainsi préparé a été appliqué sur les fentes des murs ou a été introduit, roulé en cylindre ou en cône dans l'orifice des tanières. Le contrôle et l'enregistrement du nombre de phlébotomes dans les localités explorées a été fait le jour suivant.

L'installation des entonnoirs à éprouvettes ou des papiers collants a été effectuée avant le coucher du soleil, tandis que leur contrôle s'est fait dans le courant de la matinée, suivant à près de 14 à 16 heures à partir de l'installation. Les phlébotomes ont été détachés des papiers collants par leur introduction dans un bain d'alcool à 96°, ou par l'application d'une goutte d'alcool sur l'insecte collé et par son enlèvement délicat à l'aide d'un pinceau fin, imbibé d'alcool.

### CONDITIONS DE MILIEU

Les observations de la région de Craiova ont été effectuées dans un nombre de 11 localités, parmi lesquelles 8 situées dans le bassin du Jiu et 3 sur la rive du Danube. La région explorée, située à 44° latitude N et 24° longitude E est représentée en grande partie par une zone de collines qui ne dépassent pas 150-200 m. d'altitude et qui descend vers le sud, dans la plaine du Danube. Le sol, en grande partie de constitution argilo-sablonneuse, présente de nombreuses terrasses et des bords escarpés, dûs à l'érosion des eaux, aux éboulements de terrains ou à l'extraction du sable des carrières.

*Le Climat*, continental, enregistre dans cette zone des températures moyennes au-dessus de 10°C. entre le 11.IV-22.X des températures maximales au-dessus de 25°C., 110 jours par an et 30°C, 40 jours par an. Des températures moyennes dépassant 20°C, condition

TABLEAU I. La Fréquence des Phlébotomes dans les Habitations août 1955.

Localité	Date du contrôle	Nombre des foyers		Nombre total de phlébotomes
		contrôlés	à phlébotomes	
Segarcea	14. VIII.	15	7	28
	25. VIII.	10	2	11
Dăbuleni	19. VIII.	5	4	68
Calopăr	22. VIII.	10	—	—
Bouveni	26. VIII.	8	—	—
Drănic	26. VIII.	5	—	—
	2. VIII.	5	—	—
Calafat	29. VIII.	5	—	—

nécessaire à l'évolution des phlébotomes, sont notées en général pendant les mois de juin, juillet, août.

Les particularités du sol de cette zone, le climat de steppe et la végétation existante favorisent le développement d'un grand nombre de rongeurs et d'oiseaux caractéristiques à ces biotypes. Le sol est percé de nombreuses galeries de spermophiles (*Citellus citellus*) tandis que les guépiers (*Merops*) creusent leurs nids dans les parois abruptes argilo-sablonneuses.

### LES RÉSULTATS DES OBSERVATIONS

Les conditions climato-logiques de l'année 1955, printemps tardif, été frais à précipitations abondantes, n'ont pas été favorables au développement des phlébotomes. Les premiers sondages effectués au mois de juillet, tout comme ceux ultérieurs du mois d'août, ont montré une fréquence très réduite et même leur absence dans les habitations de la plupart des localités explorées (Tableau I).

TABLEAU II. Fréquence des Phlébotomes dans les Biotopes naturels.

Localité	Date	Nombre des nids, terriers		Total	Nombre de phlébotomes capturés par nid ou terrier.	
		contrôlés	à phlébotomes		Valeurs	
					minimes	maximes
Segarcea	23. VIII.	8	6	49	1	17
	27. VIII.	20	9	46	3	7
	29. VIII.	25	16	88	1	12
	1. IX.	25	21	159	1	32
	4. IX.	25	13	23	1	5
	6. IX.	25	9	17	1	4
	8. IX.	25	15	55	1	12
	10. IX.	25	4	5	1	2
	14. IX.	24	4	7	1	1
	20. IX.	25	8	16	1	7
	22. IX.	25	3	6	1	4
	3. X.	25	1	1	1	2
	5. X.	23	6	8	1	2
	6. X.	26	1	1		
9, 13, 14, 15, 16, X.	25-27	—	—	—		
Drănic	27. VIII.	21	11	222	1	116
	2. IX.	16	15	268	1	85
	9. IX.	28	23	165	1	50
	20. IX.	21	3	7	1	4
Calopăr.	23. VIII.	2	1	12		
Bouveni	27. VIII.	4	2	29	6	23
Cerătu	7. IX.	17	4	5	1	2
Lipov	16. IX.	44	15	40	1	9
Călugărei	6. IX.	24	7	11	1	3
Carpen	6. IX.	26	2	4		2
Potelu	5. IX.	30	—	—		
Silistioara	5. X.	35	—	—		
Calafat	29. VIII.	20	—	—		
Total:				1.224		

Il faut remarquer que, dans les localités Dābuleni et Calafat, lors d'un sondage effectué entre le 4-7-IX 1952, année à été chaud et sec, on a trouvé de 30 à 145 phlébotomes par foyer, tandis qu'en 1955, dans les mêmes localités, on a trouvé dans 5 foyers contrôlés: à Dābuleni un total de 68 exemplaires et à Calafat zéro.

Dans les biotopes naturels, explorés aux environs de quelques localités, on a capturé, à l'aide des papiers collants ou des entonnoires métalliques un nombre varié de phlébotomes (Tableau II).

Dans la localité Segarcea où l'on a dépisté pendant les années 1954-1955 un cas de leishmaniose viscérale, confirmé par la mise en évidence de l'agent étiologique dans le matériel récolté par une ponction sternale, les observations ont eu un caractère périodique. Ici, dans les stations de capture: nids de *Merops* situés sur les côtes argilo-sablonneuses et dans les terriers en majorité creusés par les *Citellus citellus* dans la carrière de sable se trouvant entre les vignes à près de 4 km. de la localité, le nombre total des phlébotomes capturés augmente vers la fin du mois d'août et au début de septembre; il décroît après le 10-IX pour que, passé le 8-X on ne trouve plus aucun phlébotome, bien que les journées chaudes fussent revenues.

Il faut remarquer qu'on a trouvé des phlébotomes beaucoup plus nombreux dans les nids d'oiseaux des côtes, que dans les terriers de *Citellus citellus*. La plupart des exemplaires capturés étaient des mâles, avec une légère diminution procentuelle en faveur des femelles vers la fin du mois d'août et au mois de septembre (Tableau III). Après le 22-IX, pour un nombre restreint de phlébotomes, les femelles prédominent.

TABLEAU III. La Fréquence par Sexes dans les Stations de capture. Segarcea, août-septembre, 1955.

Date	Phlébotomes capturés		
	Nombre total	♂ %	♀ %
23. VIII.	49	97,9	2
27. VIII.	46	84,7	15,2
29. VIII.	88	80,6	19,3
1. IX.	159	91,8	8,1
4. IX.	23	82,6	17,3
8. IX.	55	87,2	12,7
10. IX.	5	60	40
14. IX.	7	85,7	14
20. IX.	16	81,2	18,7
22. IX.	6	66,66	33,3
3. X.	1	—	100
5. X.	8	37,5	62,5
5. X.	1	—	100

La prédominance des mâles dans les nids d'oiseaux et dans les terriers inhabités pour la plupart, dans la période (14. VIII-20. IX) tout comme la capture des phlébotomes dans de simples excavations, peu profondes, nous fait supposer que ces dernières sont employées surtout comme lieux d'abris. Mais, vu que parmi le grand nombre de phlébotomes capturés dans les nids d'oiseaux et dans les terriers de rongeurs, on a trouvé aussi deux femelles qui ont déposé 2 à 4 œufs sur le papier collant, à coup sûr immédiatement après la capture, il est possible que ces nids soient employés aussi pour la ponte.

On a effectué aussi quelques examens de sol, réalisés par le creusement des parois de certains nids d'oiseaux, de la catégorie de ceux où se trouvaient régulièrement de nombreux phlébotomes; on n'y a pas trouvé de larves de phlébotomes. L'examen a été effectué à la loupe binoculaire et au microscope par la méthode directe, ou avec une solution sursaturée de NaCl. Le nombre réduit d'observations relatives à ce chapitre ne permet pas encore de tirer une conclusion.

Vu que, sur les papiers collants introduits dans les nids pendant la journée (journée chaude, soleil) et retirés après 2 à 3 heures, on n'a pas trouvé de phlébotomes, tandis que peu de temps avant le crépuscule et au moment même de l'introduction de ces papiers dans les nids, on a pris quelques fois 6 à 8 phlébotomes,—on peut déduire plutôt l'absence circulation des phlébotomes pendant la journée plutôt que leur absence des nids dans cette



période de la journée. L'observation que vers le crépuscule, quelques phlébotomes ont été vu volant à l'intérieur du nid vers la sortie, exemplaires qui ont pu être facilement pris avec la capteur aspirateur, vient aussi à l'appui de cette affirmation.

Le vol au crépuscule de ces phlébotomes de l'intérieur du nid vers l'ouverture, est beaucoup plus accentué par le temps couvert. La pluie légère, la brume, ne les empêchent pas de quitter ces abris de jour; par un temps pareil ils ont pu être facilement capturés sur la végétation basse, autour des nids. Les phlébotomes identifiés ont été déterminés d'après les caractères morphologiques des mâles et des femelles. Tous les phlébotomes récoltés dans la localité Dăbuleni, habitations, tout comme la plupart de ceux de Segarcea, habitations, appartenant à l'espèce *Ph. Papatasii*.

Parmi le matériel recueilli dans la commune de Segarcea, habitations, on a trouvé aussi 4 exemplaires femelles de *Ph. perfiliewi*, trois récoltés dans deux métairies situées à l'extrémité du village, vers les champs et l'un dans une métairie isolée du village, à proximité du canton de la voie ferrée.

Dans une des habitations, on avait diagnostiqué l'année précédente un cas de leishmaniose viscérale.

Le *Phlébotomus perfiliewi* a été trouvé aussi cette année dans une collectivité, à Calopăr, localité où l'on n'a pas rencontré le *Ph. papatasii* à l'occasion du contrôle de 10 métairies. Dans cette localité, le *Ph. perfiliewi* a été capturé dans une métairie située à une des extrémités du village, métairie où l'on a dépisté en 1955 un cas de leishmaniose viscérale (confirmée par ponction sternale). Dans cette métairie, sur un papier appliqué sur une des nombreuses fentes du mur extérieur d'une chambre de dépendance, on a trouvé sur la partie du côté du mur 4 exemplaires de *Ph. perfiliewi* mâles, tandis que vers l'extérieur, du côté de la cour et des champs, une femelle appartenant à la même espèce.

Dans les recherches entreprises, on a rencontré fréquemment le *Ph. perfiliewi* en dehors des villages. C'est l'espèce trouvée dans les biotopes naturels, les terriers de spermophiles et surtout dans les nids de guépiers qui se trouvent dans les terrains argilo-sablonneux, les côtes abruptes, les parois des carrières de sable, etc. Dans les sondages effectués dans de tels biotopes situés à des distances variant de quelques centaines de mètres jusqu'à 4 à 5 km. des localités visitées, il a été trouvé dans 8 localités du bassin moyen de la rivière du Jiu: Segarcea, Calopăr, Ceratu, Lipov, Drănic, Bouveni, Călugărei, Carpen. Il n'a été trouvé qu'à la suite des recherches effectuées dans les biotypes naturels à proximité des localités Calafat, Potelu, Silișticara, situées sur la rive du Danube.

Le grand nombre (481 exemplaires) qui a été capturé dans le biotope Segarcea (la carrière de sable) et surtout 662 exemplaires dans le biotope Drănic (colline argilo-sablonneuse, à trois terrasses superposées) où l'on est parvenu, dans certains nids jusqu'à 116 exemplaires par capture (27.VIII), sur la terrasse supérieure et leur rareté dans les habitations et dans les villages, montre que c'est une espèce sauvage. Ainsi n'a-t-on pas capturé le *Ph. perfiliewi* à Drănic et Bouveni, dans les habitations du voisinage immédiate des biotopes naturels où il était très abondant. On n'a pas trouvé non plus le *Ph. papatasii* dans les biotopes naturels explorés.

## DISCUSSIONS

La présence des cas de leishmaniose viscérale dans une zone où l'on signalait, par le passé, le *Ph. papatasii* comme une espèce très abondante, espèce qui est peu incriminée par les différents auteurs (Craig et Faust 1949, Mackie, et al., 1949) de transmettre cette maladie, réclame une exploration plus minutieuse pour dépister éventuellement aussi d'autres espèces. Nos recherches, effectuées dans les conditions climatiques de l'année 1955, peu favorable au développement du *Ph. papatasii*, nous ont démontré des densités réduites ou même l'absence de cette espèce, dans l'aire où elle était très répandue en d'autres années.

L'espèce *Ph. perfiliewi*, identifiée dans la zone explorée apparaît pour notre pays comme une espèce sauvage, en tenant compte des biotopes où elle a été trouvée d'une manière fréquente et en grand nombre, tandis que dans les collectivités humaines, du moins dans les conditions climatologiques de l'année 1955, elle est rencontrée sporadiquement et seulement à la périphérie des localités! Il n'est cependant pas exclu que, dans les années à conditions climatologiques favorables, l'augmentation encore plus forte de la densité de l'espèce *Ph. perfiliewi* dans les biotopes naturels, détermine une migration plus accentuée vers les collectivités humaines, en réalisant ainsi un contact plus étroit avec l'homme.

La prévalence de l'espèce *Ph. perfiliewi* dans les collectivités humaines, est mentionnée par les auteurs italiens (Vanni 1939-40, Corradetti 1954) qui lui attribuent le rôle de vecteur principal dans la transmission de la leishmaniose cutanée dans les Monts Abruzzes (Italie). La même espèce est incriminée comme vecteur de la leishmaniose viscérale en Palestine (Mackie, *et al.*, 1949), ainsi qu'en Serbie méridionale et septentrionale (Simitch 1955).

Dans les conditions qui régissent notre pays, les caractéristiques oecologiques que nous avons notées, cette année, pour l'espèce respective, l'indique comme étant de prédominance sauvage; ses relations avec l'homme apparaissent rares et sporadiques, fait qui serait de nature à expliquer la rareté des cas et leur distribution à la périphérie des collectivités. Des recherches ultérieures auront à préciser le rôle de cette espèce dans la transmission de la leishmaniose viscérale dans notre pays.

### CONCLUSIONS

Il résulte des observations effectuées pour l'intervalle du 14.VIII. au 15.X. 1955 dans une des zones de distribution du *Ph. papatasi* (région de Craiova), zone en laquelle on a découvert en 1954 un foyer de leishmaniose viscérale, que l'on a identifié, pour la première fois dans notre pays, l'espèce *Phlébotomus perfiliewi*. Dans les conditions locales de l'année 1955—été frais, avec d'abondantes précipitations,—le *Ph. perfiliewi* apparaît comme une espèce sauvage, fréquente dans des biotopes naturels: terriers de *Citellus citellus* et surtout nids de *Merops* et rare à la périphérie des collectivités humaines.

Les terriers et les nids d'oiseaux des biotopes explorés sont utilisés en tant qu'abri de jour et, d'après certaines observations, aussi pour la retraite des femelles prêtes à la ponte.

On n'a pas rencontré le *Ph. papatasi* dans les biotopes naturels explorés.

Les endroits de développement et d'abri de l'espèce *Ph. perfiliewi* dans notre pays apparaissent comme différents de ceux cités par quelques auteurs dans d'autres pays. Nous mentionnons que cette espèce a été capturée aussi dans trois habitations où l'on a découvert dans deux d'entre elles, en 1954, des cas de leishmaniose viscérale.

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# Phlebotomus Sandflies in Panama

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## ABSTRACT<sup>1</sup>

A survey of the *Phlebotomus* sandflies in Panama was commenced in 1943 in collaboration with Dr. M. Hertig. Over 80,000 specimens of nearly 60 species were taken by 1955. Collecting methods are described and their usefulness in revealing the habits and habitats of adult sandflies assessed. Light traps yielded most specimens and the largest number of species, but did not give a true picture of relative abundance, as some species are rarely attracted to light. Stable traps with horse bait yielded good numbers of a few species. Searching of daytime resting places such as hollow trees, crevices in masonry, animal burrows, and narrow spaces between buttressed roots of forest trees gave a truer picture of favored habitats, but some species, abundant in light traps or taken biting, are rarely taken in daytime resting places. The more abundant species and those biting man are discussed. Their habits and habitat preferences may be represented graphically, giving a distinctive pattern for each species.

## DISCUSSION

W. H. R. LUMSDEN. Have any attempts been made to obtain virus isolations from *Phlebotomus* in Panama?

G. B. FAIRCHILD. Not yet. We hope that work along these lines will be possible in the not too distant future.

W. H. R. LUMSDEN. Could the outbursts of *Phlebotomus* spp. described also be due to mass emergence from pupae initiated by some climatic factor?

G. B. FAIRCHILD. We do not think so, though the possibility exists. The phenomenon is exceedingly local, affecting only one trap on any night, traps a few hundred yards away being unaffected, showing average catches for that night.

R. LEVI-CASTILLO. We have been using in Ecuador all types of traps, observing that in all of them the catches were varied. In Shannon traps and other types of animal traps females were caught in large numbers. Whereas, with traps made of paper embedded with castor oil that withstand rain, we caught more males than females. The insects adhered to the paper and were taken off by means of a drop of alcohol and a No. 4 entomological pin that served to move the specimen into 40% alcohol for preservation.

<sup>1</sup>Complete paper to be submitted to the *Annals Ent. Soc. Amer.*





# The Feeding Mechanism of *Dermacentor andersoni* Stiles

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## ABSTRACT

The structure of the capitulum of *D. andersoni* is described with reference to previous works on the mouthparts of ticks. Particular attention is given to the nature and function of the hypostomal groove, oral aperture, and pharyngeal valve. The flow and course of the salivary secretion during feeding and its probable relation to the production of paralysis in tick hosts is discussed. Artificial feeding observations suggest that in nature this secretion may be either injected into the host's tissues or swallowed directly with the inflowing blood.

## DISCUSSION

R. GEIGY. Is the saliva extruded intermittently? Do the mouth-parts pierce the blood vessels and produce a haemorrhagia under the skin?

J. D. GREGSON. Yes, a haemorrhage is produced. Since paralysis disappears so rapidly upon removal of a causative tick, it seems that saliva is injected frequently in the case of paralysis, and perhaps not at all where paralysis is not present, the saliva then being short-routed to the pharyngeal opening.

A. R. BARR. Do you consider ticks to be "blood feeders" in the same sense as mosquitoes, or do you believe they feed on all sorts of tissue fluids as well as blood?

J. D. GREGSON. There is little doubt that *D. andersoni* ingests large amounts of blood during normal feeding, because of the blood breakdown products present in the gut and excreta. However, it is likely that, since capillaries are not tapped, tissue fluids are also drawn in during feeding. Engorging nymphs of *D. albipictus* are frequently pale in colour and may have engorged on plasma alone.

P. A. WOKE. Does the severity of paralysis bear a relation to the numbers of ticks attacking?

J. D. GREGSON. Not necessarily. Cattle are usually only paralysed by several dozens of ticks, but may, on occasion, be paralysed by one female. Dogs and sheep may be paralysed by a single tick or be unaffected by as many as 50. Humans are usually, but not necessarily, paralysed by one female.



# Artificial Feeding of Ixodid Ticks for Studies on the Transmission of Disease Agents

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## ABSTRACT<sup>1</sup>

*In studies on the importance of ixodid ticks as possible vectors of various disease agents, a glass capillary tube technique modified after that of Chabaud was successfully used for the experimental infection of *Dermacentor andersoni* and *Amblyomma maculatum* with *Leptospira pomona*. The technique briefly is as follows: The ticks are immobilized by partially embedding them in a ridge of plasticine on an ordinary microscope slide. Glass capillary tubes, 35.0 to 1.1 mm., with one end drawn to suitable fineness are filled by capillary action with infectious suspensions and the fine ends of these tubes are then pushed over the piercing mouth-parts of the ticks. Blocks of plasticine are used not only to hold the capillaries in position but also as operating tables for the manipulation of the tubes, a process which has to be carried out under low power magnification. The ticks feed readily at room temperature for a period of 4 to 6 hours, during which they ingest approximately 0.01 to 0.03 ml. of fluid. Thus, 75 *D. andersoni* and 25 *A. maculatum*, males and females, were induced to ingest a suspension of *L. pomona* in Verwoort's medium. When the ticks stopped feeding, they were removed, stored for 14 days, and then placed upon weanling guinea pigs to complete engorgement. Several of these animals subsequently developed leptospirosis, indicating survival of leptospirae in, and transmission of this organism by both species of ticks. The described technique provides an excellent artifice for the experimental infection of ixodid ticks with viruses and other pathogens.*

<sup>1</sup> Complete paper published in *Journal of Infectious Diseases* 100: 212-214. 1957.





# Tick Paralysis in the Dog: A Neurophysiological Study<sup>1, 2</sup>

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## ABSTRACT

Tick paralysis was produced in mongrel dogs by applying the tick *Dermacentor andersoni* Stiles. Paralysis occurred in 9 out of 23 trials when approximately 10 female and 10 male ticks were applied to each animal. The anterior tibial muscle contracted when stimulated directly but not when stimulated through the peroneal nerve. As motor nerve fibre conduction was demonstrated to be present, this implied that the paralysis was due to failure in transmission at the neuro-muscular junction. The responses of the muscle to intra-arterial injections of acetylcholine, to antagonists of blocking agents and to repetitive stimulation indicated that the paralysis did not resemble that produced by blocking agents or anticholinesterases but was similar to botulin toxin poisoning. The flexor reflex, which was measured by recording the reflex potentials in the peroneal nerve when a stimulus was applied to the popliteal nerve, demonstrated that there was also a disturbance of transmission in the spinal cord. The site of action of the tick 'toxin' has been located at the neuro-muscular junction and probably at some synapses in the spinal cord. The evidence indicates that the mechanism of action is probably a decreased liberation of acetylcholine at these sites.

## INTRODUCTION

Tick paralysis is an acute ascending paralysis which may terminate fatally due to respiratory arrest. This disease is known to occur in North America, Australia, Europe, and South Africa and affects domestic animals and humans (Abbott, 1943).

As most cases of tick paralysis in North America have been caused by *Dermacentor andersoni* Stiles, the disease has been largely confined to the northwestern United States and the southwestern region of Canada. More recent records of cases in the eastern and southern United States, due largely to *D. variabilis* Say (Costa, 1952), indicate a more widespread distribution of this disease than is generally recognized. The disease merits attention not only on account of the loss of livestock but more particularly because of the deaths of humans. To date, over 300 human cases, with a mortality of 12 per cent, have been recorded on this continent (Rose, 1954). The fatalities could be eliminated in the future by early recognition of the disease and by prompt removal of the tick.

Research on this problem in the past has been essentially concerned with the production of paralysis in experimental animals (Hadwen & Nuttall, 1913) and with the effects of extracts of the salivary glands of ticks (Ross, 1926) or of their eggs (Steinhaus, 1942). The aim of this investigation was to locate the site and to determine the pathological mechanism of the paralysis. The neuro-physiological study was carried out on mongrel dogs which were paralysed by applying the ticks, *D. andersoni* Stiles. A previous investigation had shown that tick paralysis could be induced in this animal for experimental study (O'Rourke and Murnaghan, 1954). The cause of tick paralysis is generally accepted to be due to a neuro-toxin which is secreted by the salivary glands of the feeding female tick (Gregson, 1952), and mating has been shown to increase its feeding rate (Gregson, 1944). As the latter might result in a greater production of neurotoxin, both female and male ticks were applied.

## METHODS

The ticks were kindly supplied by J. D. Gregson, Entomology Laboratory, Canada, Department of Agriculture, Kamloops, B.C. They were dispatched by air express to Ottawa and stored in the refrigerator until required.

<sup>1</sup>Aided by a grant from the Department of Agriculture, Canada.

<sup>2</sup>Since this paper was submitted for publication further study (Murnaghan, M. F. 1958. Neuro-anatomical site in tick paralysis, *Nature* 181: 131) has shown that the paralysed muscle exhibits an increased sensitivity to acetylcholine injected intra-arterially and that end-plate potentials are absent during nerve stimulation. These findings substantiate the suggested hypothesis that the paralysis is due to failure in the liberation of acetylcholine at the somatic motor nerve terminals.

The hypothesis has recently been conclusively proven by the author (unpublished observations) as it has been found that when the anterior tibial muscles of 5 paralysed dogs were perfused with Ringer's solution, acetylcholine was not liberated when the peroneal nerve was stimulated.

Finally, due to an improvement in technique in applying the ticks, paralysis could be produced in at least 90% of the animals.

The dog was kept in a cage which rested above a tray containing detergent solution. The ticks, usually 10 females and 10 males, were applied on the back of the neck of the dog by means of a small dome-shaped wire capsule fastened to the hairs by cement. A small pledget of moist cotton was included to avoid desiccation of the ticks. In an attempt to prevent the dog scratching off the neck capsule until the ticks had time to attach, it was injected subcutaneously with morphine sulfate, 10 mgm./kgm., twice a day for three days.

Neurophysiological studies were carried out on normal and paralysed dogs. Anaesthesia was induced with ether and maintained with chloralose, 80 mgm./kgm. intravenously. The tendinous insertion of the anterior tibial muscle of one leg was isolated and connected to an isometric lever. Drill bits were inserted into the lower ends of the femur, and tibia and fibula, to maintain fixation of the limb. The femoral nerve was cut and the sciatic nerve and its subdivisions, the peroneal and popliteal, were freed from the surrounding tissues and laid on silver-silver chloride electrodes for stimulation or recording. In some experiments the anterior tibial muscle was prepared for close arterial injection by the method described by Brown (1938). In order to investigate conduction in motor and sensory nerve fibres the lower lumbar spinal roots were exposed and laid on silver-silver chloride electrodes.

A Grass S4 Stimulator was used throughout this study which permitted simultaneous variation in strength, duration and frequency of the stimulus. A Grass Isolation Unit isolated the stimulus from ground. The maximal strength of stimulus required for nerve was approximately 1 volt; 100–150 volts were used for direct muscle stimulation. The pulse durations were 0.2 and 2 milliseconds respectively. Nerve action potentials were visualized and photographed on a cathode-ray screen after A.C. amplification.

## RESULTS and DISCUSSION

### INCIDENCE OF PARALYSIS AFTER TICK APPLICATION

Table I summarizes the results obtained in 23 trials on 14 dogs. Paralysis occurred 9 times, being complete in 5 and incomplete in 4. The terms complete and incomplete, used in this paper, refer to the degree of paralysis of the anterior tibial muscle to indirect stimulation. This flexor muscle was chosen for study because it had been found that it was consistently involved in the early stages of the paralysis. The extensor muscles (gastrocnemius, quadriceps), however, were more resistant, as the crossed extensor reflex was present in some cases when the flexor withdrawal reflex was absent. In two trials the paralysis was transient due to some of the ticks falling off. In one of these cases recovery occurred in 5 hours. Paralysis appeared in 7–9 days and approximately 8 engorged female ticks were required to produce it. In the 14 trials where paralysis did not occur, the dog scratched off the neck capsule and ticks in 8 cases, the ticks failed to feed in 5, and in one the cause was unknown because 8 engorging females failed to produce paralysis. In one of the 5 cases where the ticks failed to feed, the dog died of an intercurrent infection which was not identified.

### NEUROPHYSIOLOGICAL STUDY ON DOGS

#### (a) LOCATION OF THE PARALYSIS AT THE NEUROMUSCULAR JUNCTION

The first significant phenomenon observed (Murnaghan, 1955) was that the anterior tibial muscle failed to contract on stimulating the peroneal nerve but responded to direct stimulation (Fig. 3). Rose and Gregson (1956) have recorded a similar observation. This finding implied that either, (1) the motor nerve fibres in the peroneal nerve failed to conduct the nerve impulse, or (2) there was a block at the neuro-muscular junction.

In order to investigate the first hypothesis, the lower lumbar spinal roots were exposed in a dog where all the muscles of the leg were insensitive to nerve stimulation. This degree of paralysis was necessary in order to exclude the possibility that some fibres in the peroneal nerve would be conducting if the absence of nerve conduction was assumed to be the etiological factor. When the 6th lumbar ventral root was stimulated with a single shock, a nerve impulse was recorded from the peroneal nerve (Fig. 1a) indicating that conduction was present in the motor nerve fibres. In the same experiment conduction was shown to be also present in sensory nerve fibres (Fig. 1b) by recording from the 7th lumbar dorsal root and stimulating the popliteal nerve. The latter finding confirmed the assumption from previous experiments that the sensory fibres did conduct, as it had been noted that a paralysed dog, when lightly anaesthetized, often responded by crying or with laboured respiration during sensory nerve stimulation.

TABLE I—Summary of Results of Tick Application in Dogs.

Dog No.	Weight kgm.	Trial No.	Date	No. of ticks applied		Paralysis of anterior tibial muscle	Days until paralysis occurred	No. of female ticks engorged	Cause of failure to produce paralysis	Fate of dog
				M.	F.					
1	9	1	23/4/55	10	10	-	-	2	Neck-capsule off	-
		2	9/5/55	10	10	-	-	0	" "	-
		3	14/5/55	10	10	-	-	1	" "	-
		4	24/5/55	10	10	-	-	1	Ticks not feeding	-
2	8	1	27/4/55	10	10	Incomplete	8	7	-	Normal next day 5 ticks feeding
		2	9/5/55	10	10	Complete	9	6	-	Removed ticks Normal next day
3	4	3	24/5/55	10	10	Complete	7	7	-	OPERATED
4	5	1	24/5/55	10	10	Complete	7	6	-	OPERATED
5	9	1	1/6/55	10	10	Incomplete	8	8	-	OPERATED
6	8.5	1	12/9/55	10	6	-	-	1	Neck-capsule off	-
7	8.5	1	17/4/56	10	10	-	-	1	Neck-capsule off	-
8	7.5	1	17/4/56	10	10	Incomplete	8	6	-	OPERATED
9	9.5	1	24/4/56	10	10	Complete	8	9	-	OPERATED
10	12	1	24/4/56	10	10	Complete	7	9	-	OPERATED
11	14	1	3/5/56	10	10	-	-	8	?	-
		2	12/5/56	7	13	-	-	0	Ticks not feeding	-
12	8	1	9/5/56	5	10	Incomplete	9	10	-	Normal in 5 hr. 7 ticks feeding
13	11.5	2	22/5/56	15	16	-	-	0	Neck-capsule off	-
		3	29/5/56	10	15	-	-	0	" "	-
		4	26/6/56	10	18	-	-	0	Ticks not feeding	-
14	11.5	1	21/5/56	10	10	-	-	0	Ticks not feeding	-
15	8.2	1	21/5/56	10	10	-	-	0	Ticks not feeding	Died on 8th day
16	7	1	26/6/56	8	18	-	-	1	Neck-capsule off	-



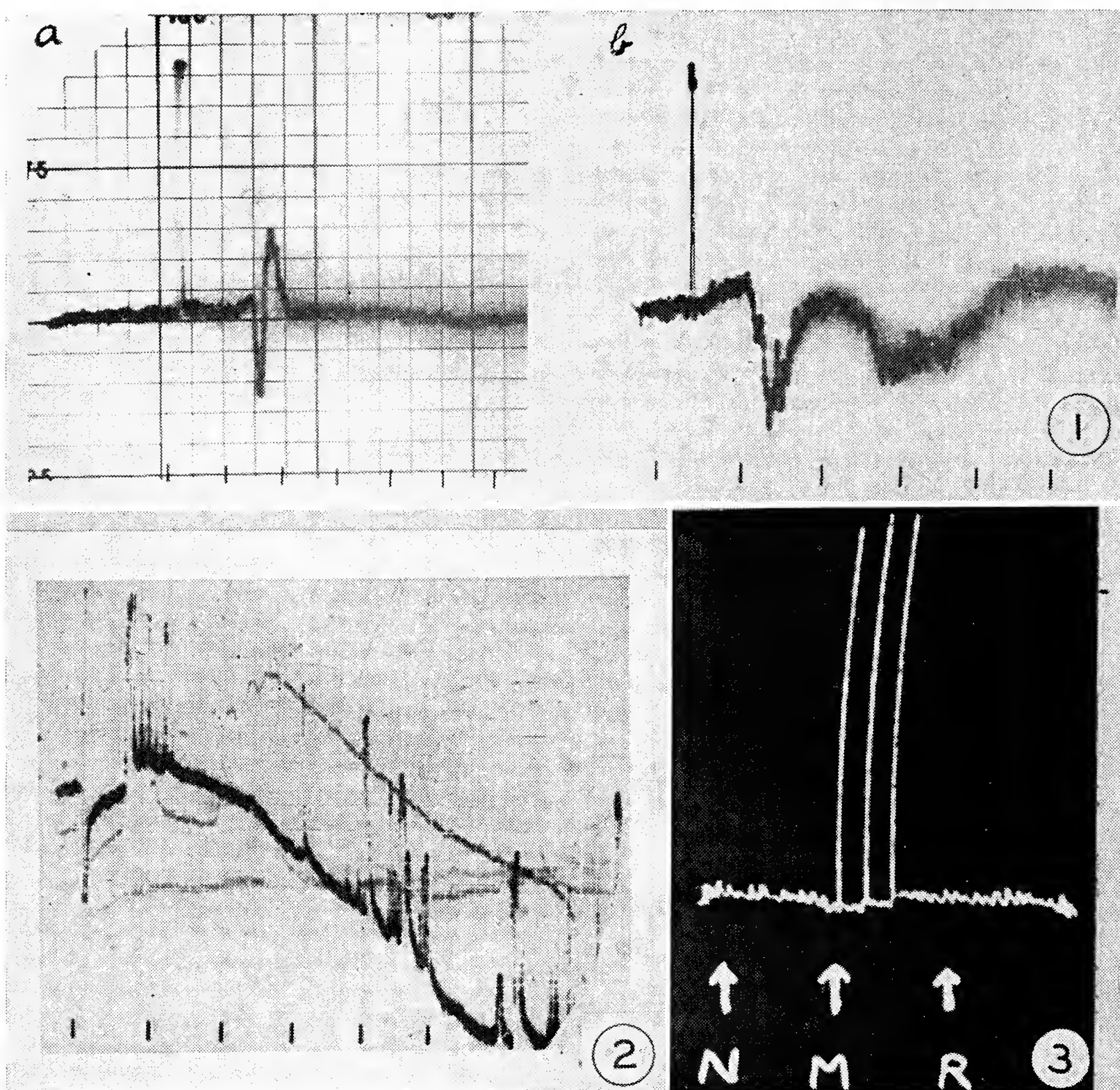


Fig. 1. (a) Conduction in motor nerve fibres: artifact and nerve impulse; time scale, 2 milliseconds. (b) Conduction in sensory nerve fibres: artifact to the left; time scale, 5 milliseconds.

Fig. 2. Reflex potentials recorded from the peroneal nerve (complete paralysis): artifact to the left. Time scale, 20 milliseconds. Note prolonged after-discharge and response from previous stimulus due to long-persistent cathode-ray screen.

Fig. 3. Responses of anterior tibial muscle (complete paralysis): stimulation of peroneal nerve (N), muscle (M) and popliteal nerve (R).

Having excluded the inability of the motor nerve fibres to conduct as the cause of the paralysis, the mechanism of the neuro-muscular block required elucidation. In an initial study (Murnaghan, 1955) it had been found that when 200 microgm. of acetylcholine was injected rapidly into the femoral artery of a dog, in which the anterior tibial muscle failed to respond to nerve stimulation, the muscle responded by a contraction of approximately two seconds duration. Although the dose of acetylcholine was large, the amount reaching the anterior tibial muscle must have been a small fraction of the total. In a later study the use of the technique of close arterial injection permitted an assessment of the sensitivity of the motor end plates to acetylcholine. Unfortunately, the only paralysed dog available for investigation was one in which the anterior tibial muscle was incompletely paralysed. This is illustrated in Figure 4A where the contractions were less to indirect as compared to direct stimulation. Increasing doses of acetylcholine, 2.5, 10 and 40 microgm., caused increasing heights of contraction (Fig. 4B) and these responses were similar to those of a normal muscle.

This normal sensitivity to acetylcholine of the motor end-plates indicated that the paralysis was not due to a curare-like substance. This was substantiated by the results on



tetanzation. Wedensky inhibition did not appear during indirect rapid repetitive stimulation of the incompletely paralysed anterior tibial muscle and subsequent post-tetanic block did not occur. On the contrary, the tetanus and the post-tetanic potentiation (Fig. 4E, F) were similar in all respects, except for the diminished tension, to those of direct stimulation and of indirect or direct stimulation of normal muscle. Furthermore, the responses to dual stimuli were normal in character (Fig. 4D). Finally, the increase in tension produced by neostigmine (Fig. 4C) was similar to that seen in the normal muscle but less than in the curarized one.

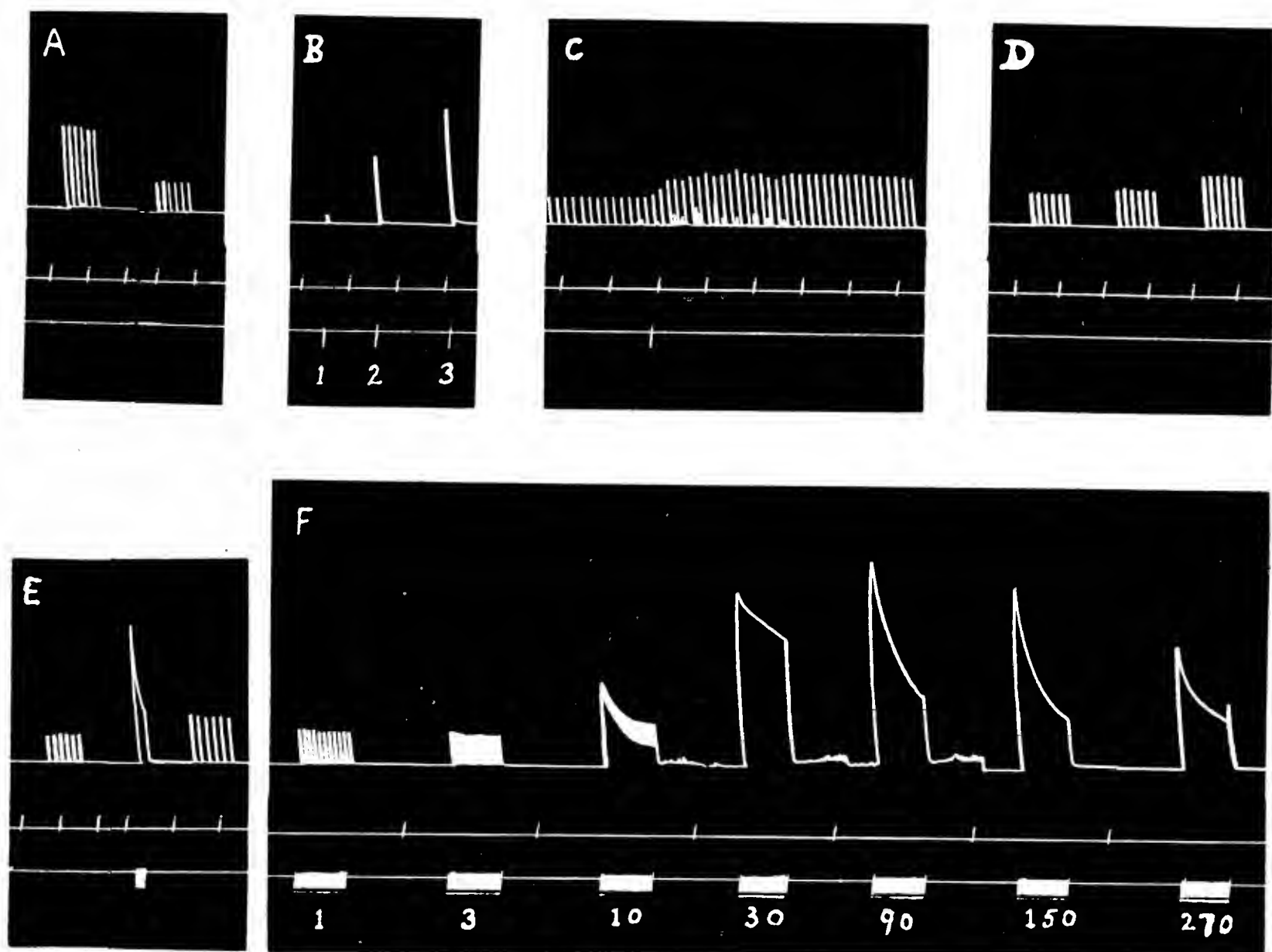


Fig. 4. Contractions of anterior tibial muscle (incomplete paralysis): peroneal nerve stimulation except in B and first-half of A. Middle record: time scale, 1 minute. Lower record: signal. (A) Direct and indirect stimulation. (B) Close arterial injections of acetylcholine: at signal 1, 2, and 3—2.5, 10, and 40 microgm. respectively. (C) Close arterial injection of 100 microgm. neostigmine at signal. (D) Dual pulses at 1, 3, and 5 millisecond intervals. (E) Tetanus, 1000 stimuli per second for 15 seconds, preceded and followed by twitches at 1 per 10 seconds. (F) Tetanus for 15 seconds at designated stimulation rates per second.

A further possibility requiring investigation was that a blockade of the depolarizing type, such as is produced by decamethonium or succinylcholine, was responsible for the paralysis. The intra-arterial injection of pentamethonium, however, failed to improve the contractions, while neostigmine increased them; this was the opposite to what would be expected if blockade was due to a depolarizing agent. Finally, neuro-muscular blockade due to cholinesterase inhibitors could be excluded because neostigmine did not increase the paralysis and the muscle maintained a tetanus during indirect stimulation.

Apart from blocking agents and cholinesterase inhibitors, the only other mechanism which is known to interfere with transmission at the neuro-muscular junction is failure in the liberation of acetylcholine. This mechanism, therefore, is probably responsible for the neuro-muscular block in tick paralysis. Such a hypothesis is substantiated by the evidence that the properties of this block and that produced by the botulin toxin (Burgen, Dickens and Zatman, 1949) are similar. There are three possible ways by which the release of acetylcholine may be diminished at the neuromuscular junction: (a) Conduction-block in the fine terminal unmyelinated nerve fibres, as is produced by the botulin toxin. (b)

Inhibition of the synthesis of acetylcholine at the nerve endings. (c) Inability of the nerve impulse to liberate acetylcholine from its non-diffusible store at the nerve terminals. Further investigation will be required to determine whether the factor responsible for inhibition of acetylcholine release is identical in the two types of paralysis.

The 'toxin' in tick paralysis differs from the botulin toxin in that its effect is not persistent, as recovery generally occurs in a short time after removal of the ticks. This suggests that the 'toxin' is either destroyed or excreted rapidly. As it has been shown that the magnesium ion, which has a transient effect, acts primarily at the neuromuscular junction by inhibiting acetylcholine release, and that the calcium ion antagonizes this effect (Del Castillo and Engback, 1954), calcium chloride was injected intra-arterially and intravenously but failed to improve neuro-muscular transmission.

The 'toxin' responsible for tick paralysis not only remains unidentified but also has not yet been isolated. Several workers have recorded symptoms in animals produced by the injection of extracts of the eggs or salivary glands of ticks. It is premature to assume that these symptoms were due to tick paralysis until a study of transmission at the neuro-muscular junction confirms such a supposition.

#### (b) THE EFFECT OF THE TICK 'TOXIN' ON CENTRAL TRANSMISSION

The possibility that the 'toxin' also acted on the central nervous system, as a contributing factor in the production of the paralysis, could not be excluded because of the similarity in physiological properties of the neuro-muscular junction and the central synapses. Central transmission was therefore investigated by means of the flexor withdrawal reflex which was elicited by stimulation of the popliteal nerve. The contractions of the anterior tibial muscle could not be used as an indicator of reflex activity because of the failure in transmission at the neuro-muscular junction (Fig. 3). Consequently, the reflex action potentials were recorded from the peroneal (or sciatic) nerve. In one experiment where the muscles of all four limbs were completely paralysed, no reflex action potentials were obtained. In this case, however, the recording equipment was inadequate so that this finding is probably of no significance. When this deficiency had been corrected, abnormal reflex action potentials occurred in a dog where the anterior tibial muscle was completely paralysed but the contralateral extensors responded to the reflex stimulation. The potentials, after a single stimulus to the popliteal nerve, consisted of multiple monosynaptic and polysynaptic forms which lasted for several seconds (Fig. 2), instead of the normal polysynaptic pattern of approximately ten milliseconds duration. In a third dog, where the anterior tibial muscle was incompletely paralysed, the reflex action potentials were normal in character.

The sustained repetitive firing of the motoneurons in the second dog was probably due to a long-lasting depolarization of these cells. There are two possible mechanisms by which this could have occurred. The motoneurons may have been in a state of partial depolarization, similar to that produced by a subliminal direct current (Fuortes, 1954), which prolonged the response to orthodromic stimulation. This state of partial depolarization probably was produced by repetitive firing from the annulo-spiral endings in the stretched paralysed muscle, or by loss of inhibition from higher centres. The second alternative mechanism is based on the assumption that its etiology is similar to that responsible for the disturbance in transmission at the neuro-muscular junction, i.e. failure in the liberation of acetylcholine. Although the role of acetylcholine in central synaptic transmission remains equivocal, recent evidence suggests that only every alternate neurone is cholinergic (Feldberg, 1950). Consequently, acetylcholine is probably not the normal humoral stimulant for the anterior horn cells. On the contrary, recent studies indicate that these cells may be inhibited indirectly by acetylcholine. Eccles, Fatt and Koketsu (1954) have presented evidence indicating that acetylcholine is liberated at the terminals of the axon collaterals of anterior horn cells and stimulates Renshaw's cells, which in turn inhibit the activity of the anterior horn cells. If the tick 'toxin' inhibits the liberation of acetylcholine at the terminals of motor nerve fibres, it is therefore logical to assume that it will also inhibit its liberation at the terminals of the collaterals of these fibres. Such an assumption might also apply to the botulin toxin. The loss of this possible normal negative feed-back system, alone or in combination with other mechanisms, may account for the prolonged after-discharge.

## HISTOLOGICAL EXAMINATION OF THE SPINAL CORD

The lumbar region of the spinal cord was examined in two paralysed dogs (Nos. 7 and 9) by Dr. J. Auer, Professor of Anatomy, University of Ottawa. The details of the findings will be published elsewhere. A brief summary is as follows:

- (a) No pathological changes could be detected in the anterior horn cells.
- (b) The terminal boutons appeared to be enlarged when compared with normal material.

The apparent enlargement of the boutons may be responsible for the disturbance in central transmission or, more probably, it may be a secondary phenomenon due to repetitive firing in the Ib nerve fibres as a result of stretching of the annulo-spiral endings in the paralysed muscle. Eccles (1953) has suggested, as an explanation for post-tetanic potentiation, that repetitive stimulation of sensory nerve fibres may cause swelling of the boutons.

## THE RELATIONSHIP BETWEEN THE NEURO-MUSCULAR BLOCK AND THE DISTURBANCE IN CENTRAL TRANSMISSION

The results indicate that the tick 'toxin' inhibits transmission at the neuro-muscular junction but appears to exert an opposite effect on transmission in the spinal cord. This difference is possibly due to the opposite roles played by acetylcholine at the two sites. While the acetylcholine liberated at the terminals of the motor nerve fibres is responsible for the transmission at the neuro-muscular junction, one synaptic pathway in the spinal cord definitely known to be cholinergic normally exerts an inhibitory effect on the motoneurons via Renshaw's cells. Inhibition in the liberation of acetylcholine at these respective pathways probably accounts for the apparent discrepancy in transmission.

The probable secondary phenomenon of the paralysis, the repetitive firing of the annulo-spiral endings in the stretched paralysed muscle, would also facilitate transmission in the spinal cord. Consequently, the motoneurons would fire repetitively which, in turn, could facilitate neuro-muscular transmission. The central disturbance in tick paralysis may therefore be regarded as a compensatory mechanism to facilitate transmission at the blocked neuro-muscular junction.

## ACKNOWLEDGEMENT

I am very grateful to Mr. Michael MacConaill for his technical assistance.

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# Some Effects of Varying Temperature and Humidity on the Lone Star Tick (*Amblyomma americanum* Linnaeus)

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## ABSTRACT

Engorged female ticks were collected and placed at 85%, 67%, and 56% relative humidity at 100°, 80°, 60°, and 50°F. At 100°F. some oviposition occurred at 85% and 67% relative humidity; none at 56%. At 80°F. oviposition and hatching occurred at all humidities but mortality of larvae was rapid at 56% relative humidity. At 60°F. oviposition was prolonged at all humidities and the eggs darkened rapidly at 56%. At 50°F. the females were completely immobile and no oviposition occurred. After varying periods of time at the above exposures, some were removed to 86°F. and 85% relative humidity. None of the females from 100°F. recovered and none of the eggs hatched. Only a partial hatch occurred from eggs exposed to 80°F. and 56% humidity. One egg mass from 60°F. and 56% hatched and the larvae fed. Oviposition began when females were removed from 50°F. and eggs hatched normally. Mold growth was rapid and destroyed some eggs. Engorged larvae survived 100°F. and all humidities for not more than 14 days; newly molted nymphs survived 11 days at 100°F. and 85%; the engorged nymph survived 25-40 days. One engorged nymph molted at the same exposure; all others failed to molt.

## INTRODUCTION

Results from a previous study (Lancaster and McMillan, 1955) at 86°F. indicated that at a relative humidity of 69 per cent eggs failed to hatch, unfed larvae survived only a few days, and engorged larvae did not molt. The results reported here are from additional work along similar lines.

## METHODS

Desiccator jars were used as humidity chambers. Relative humidities of 85, 67, and 56 per cent were acquired through use of potassium hydroxide. Buxton (1931) states that "the relative humidity for a given solution of potassium hydroxide is constant irrespective of temperature". A series of these humidities were maintained in constant temperature cabinets at 100°, 80°, 60°, and 50°F. A separate cabinet was maintained at 86°F. and all humidity chambers at 85 per cent.

Engorged females were collected from cattle and placed in glass tubes. The ends of the tubes were screened to retain hatching larvae but free circulation of air was permitted. Nine females in holding tubes were placed at each humidity at the four temperatures. This was a total of 108 engorged females in the experiment. These were placed in the chambers on May 16. All were held in the same chambers at constant temperatures until June 27. Then one female from each humidity at the different temperatures was transferred to the cabinet at 86°F. and 85 per cent relative humidity. This was repeated weekly through August 1.

Data on the survival of engorged larvae, unfed nymphs, engorged nymphs and unfed adults at the various temperatures and humidities were obtained.

## RESULTS

A general summary of the principal results are presented in Table I.

A hundred degrees Fahrenheit is too great for survival of the engorged female at relative humidities up to 85 per cent. It is doubtful that survival would occur even at a higher humidity. Twenty-four day exposures at 56 and 67 per cent were fatal and these were likely dead at an earlier date. Engorged larvae survived not more than 7 days at 56 and 67 per cent and 14 days at 85 per cent. None molted to the nymphal stage. Newly molted nymphs lasted from 4 to 11 days at the lowest and highest humidity. Engorged nymphs survived exposures of up to 40 days at 85 per cent. The range was 6 to 16 at 56; 13 to 22 at 67 and 25-40 at 85. Only 4 molted to the adult stage at 85 per cent. Unfed adults withstood 100°F. and 85 per cent humidity up to 37 days, and only 4 to 11 days at 56 and 67 per cent.

TABLE I. Results of Exposure of Engorged Female Lone Star Ticks to Different Temperatures and Humidities.

Days at:					
Temperature	Relative Humidity Percent			Optimum	
					Principal Results
100°F.	56	67	85	86°F. 85%	
	51	51	51	30	Females dead.
	60	60	60	20	Females dead.
80°F.	51	51	—	34	Some hatch, larvae died.
	60	60	—	40	No hatch.
	65	—	—	48	No hatch.
	72	—	—	41	No hatch.
	79	—	—	34	No hatch.
	85	—	—	37	No hatch.
60°F.	51	51	51	38	Oviposition, hatch, larvae fed.
	60	60	60	30	Oviposition, hatch, larvae fed.
	65	65	65	31	Oviposition, hatch, larvae fed.
	72	72	72	31	Fresh eggs*, some hatch.
	79	79	79	30	Fresh eggs*, hatch, larvae fed.
	85	85	85	42	Fresh eggs*, hatch, larvae fed.
	92	—	92	27	No hatch.
	106	—	106	14	No hatch.
50°F.	51	51	51	38	No oviposition, oviposition*, hatch, larvae fed.
	60	60	60	37	No oviposition, oviposition*, hatch, larvae fed.
	65	65	65	37	No oviposition, oviposition*, hatch, larvae fed.
	72	72	72	31	No oviposition, oviposition*, hatch, larvae fed.
	79	70	79	34	No oviposition, oviposition*, hatch, larvae fed.
	85	85	85	37	No oviposition, oviposition*, hatch, larvae fed.
	—	92	—	17	No oviposition, oviposition*, hatch, larvae fed.
					No eggs, female died.

\*When tranferred to 86°F. and 85% RH.

The lower humidity levels at 80°F. prevented hatching of the eggs after 60 days exposure. Larvae emerging from eggs exposed for 51 days died soon after hatching. Some engorged larvae molted when subjected to all humidities. Engorged nymphs survived up to 20 days at 85 and only 6 days at 56 per cent. Seventy to 100 per cent molting occurred. Newly molted nymphs survived 14 to 21 days at 56 and 85 per cent. One unfed adult lasted 132 days at 85 per cent. Some died in 8 days at 56 per cent. The range was 8 to 26 days at 56; 22 to 40 days at 67; and 27 to 132 days at 85 per cent.

Some oviposition occurred at 60°F. with oviposition accelerated when transferred to 86°F. and 85 per cent humidity. These eggs hatched and larvae lived until removed for feeding. No hatch occurred after 92 and 106 days exposure to 56 and 85 per cent humidity. Engorged larvae survived 67 and 78 days at 56 and 67 per cent humidity respectively. Molting occurred at 85 percent. Engorged nymphs survived 118 days at 67 per cent. Only two, however, molted to the adult stage and these at 85 per cent level. The unfed adult survived up to 212 days at 85 per cent and 23 to 77 days at 56; at 67 the range was 20 to 84.

No oviposition occurred at 50°F. This was very striking. Females can be kept for relatively long periods and, when placed at 86°F. and 85 per cent humidity, produce eggs which hatch. This has been made use of in distributing the laboratory work when handling large numbers of ticks. Engorged larvae survived up to 81 days at 56 and 67 per cent humidity. Newly molted nymphs survived up to 166 days at 85 per cent and the engorged nymph

up to 182 days at the same humidity level. The unfed adult survived 218 days at the 85 per cent level.

Excessively high temperature as well as low humidity can be detrimental to lone star tick survival. As the temperature is decreased survival even at the lower humidity levels is increased. Oviposition is slowed at 60°F. and does not occur at 50°F.

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### DISCUSSION

A. RALPH BARR. From the standpoint of the repeatability of results don't you think that mean survival time would be more precise than maximum survival time?

J. L. LANCASTER. Yes, but we are more interested in the maximum survival time.

As there was no further discussion the chairman invited general comments. At Dr. Twinn's suggestion, Dr. Brown briefly reviewed the resistance problem.

A. W. A. BROWN. Resistance to insecticides is now present in 32 insect species of public health importance; in 17 of these it has been fully substantiated by proofs and probably only about 5 instances are questionable. In 1951, at the time of the last Congress, malaria control was proceeding with great success. In 1956 it is still proceeding successfully, but, physiological resistance has appeared definitely in 5 species of *Anopheles*. In 1951 the discovery of DDT-resistance in Korean body lice was an isolated novelty. In 1956 DDT-resistance is present in many countries and BHC-resistance is now appearing in Japan and elsewhere. The appearance of resistance seems to be almost inevitable where insecticides exert Darwinian selection. One would like to urge that medical entomologists be on the alert for this phenomenon, measure it by quantitative test, and report it in the literature as soon as possible after the event.





# Taxonomic and Ecological Significance of Eggs of *Aedine* Mosquitoes<sup>1</sup>

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## ABSTRACT

*Eggs of aedine mosquitoes vary in size, outline, color, and chorionic detail according to genus, subgenus, group and even species in most instances. Whole eggs show sufficient specific details to permit rapid separation to species when examined in intense reflected light. Surface details of the chorion show patterns that are readily recognizable when examined as cleared material in suitable transmitted light. Taxonomists will find additional support for phylogenetic concepts of the Culicidae. Sanitarians and ecologists will be able to apply the information to extension of procedures for surveying populations of floodwater mosquitoes.*

Interest in Nearctic aedine mosquitoes is increasing because of their annoyance to humans and because of their ability to serve as vectors and reservoirs of agents causing disease. Unfortunately, little is known about these mosquitoes because most of them are difficult to colonize in the laboratory. Incomplete knowledge concerning the egg stage has hampered investigation. Satisfactory methods for finding eggs in natural sites have become known only recently. Means for recognizing eggs have been unknown until recently. Lastly the sequence of events essential for hatching the eggs is at best only partially understood. During the past four years significant advances have been made in all of these areas. The object of this paper is to point out certain of these developments.

Aedine mosquitoes spend most of their lives in the egg stage. Many species live for a year or more as eggs in mats of plant debris or in cracks in the soil. The usual sites are depressions in the general surface of the ground subject to flooding by rains, melting snow, or other transient water. Eggs bearing latent embryos may withstand drying, cold and submergence without injury for months or even a year (Horsfall, 1956). The chorion acts as a barrier to the passage of water while retaining its permeability to oxygen. The proper sequence of events for hatching, including submergence in water at hatching temperature varies considerably according to species.

Eggs used in the present work were obtained from several sources. (1) Those from natural oviposition sites were collected by washing and screening away associated coarse soil and debris followed by flotation in saturated salt solutions (Horsfall, 1956a). Separation of eggs from a sample can be accomplished in less than 15 minutes with a dependably high degree of accuracy. (2) Others were obtained from masses deposited in the laboratory by wild-caught females. These engorged females were anaesthetized, identified and segregated according to species into separate cages or individual vials where eggs were later deposited on moist cellucotton. (3) Eggs of only a few species were obtained from caged colonies. (4) Lastly, some eggs were obtained by dissection of dried, gravid females from museum collections. Soaking the dried, gravid specimens or abdomens from them in a solution of trisodium phosphate restored the eggs to their original size and shape. Chorionic sculpturing and other characters of diagnostic value were present and recognizable upon dissection.

Methods for specific determination of aedine eggs are rapid and simple. Distinctive details of whole eggs may be observed easily at magnifications of 100 diameters by placing eggs in water over a nonreflective background and illuminating them with an intense beam of white light. Cleared fragments of chorion provide excellent characters for specific identification when seen in transmitted light. The technique reported by Craig (1955) requires preliminary bleaching of the shell with hydrochloric acid and potassium chlorate. When mounted flat as single sheets and viewed with phase-contrast illumination, these bleached fragments show many distinctive features.

<sup>1</sup>Supported in part by a grant from the United States Public Health Service.

Eggs of 42 of the 61 species of Nearctic *Aedes*, as well as 8 of the 13 Nearctic *Psorophora* have been examined in detail. Among them are representatives of all subgenera and most of the smaller categories. Eggs of several *Aedes* have been described and illustrated by Horsfall and Craig (1956) and by Craig (1956). Eggs of several species of *Psorophora* have been described by Horsfall *et al.* (1952).

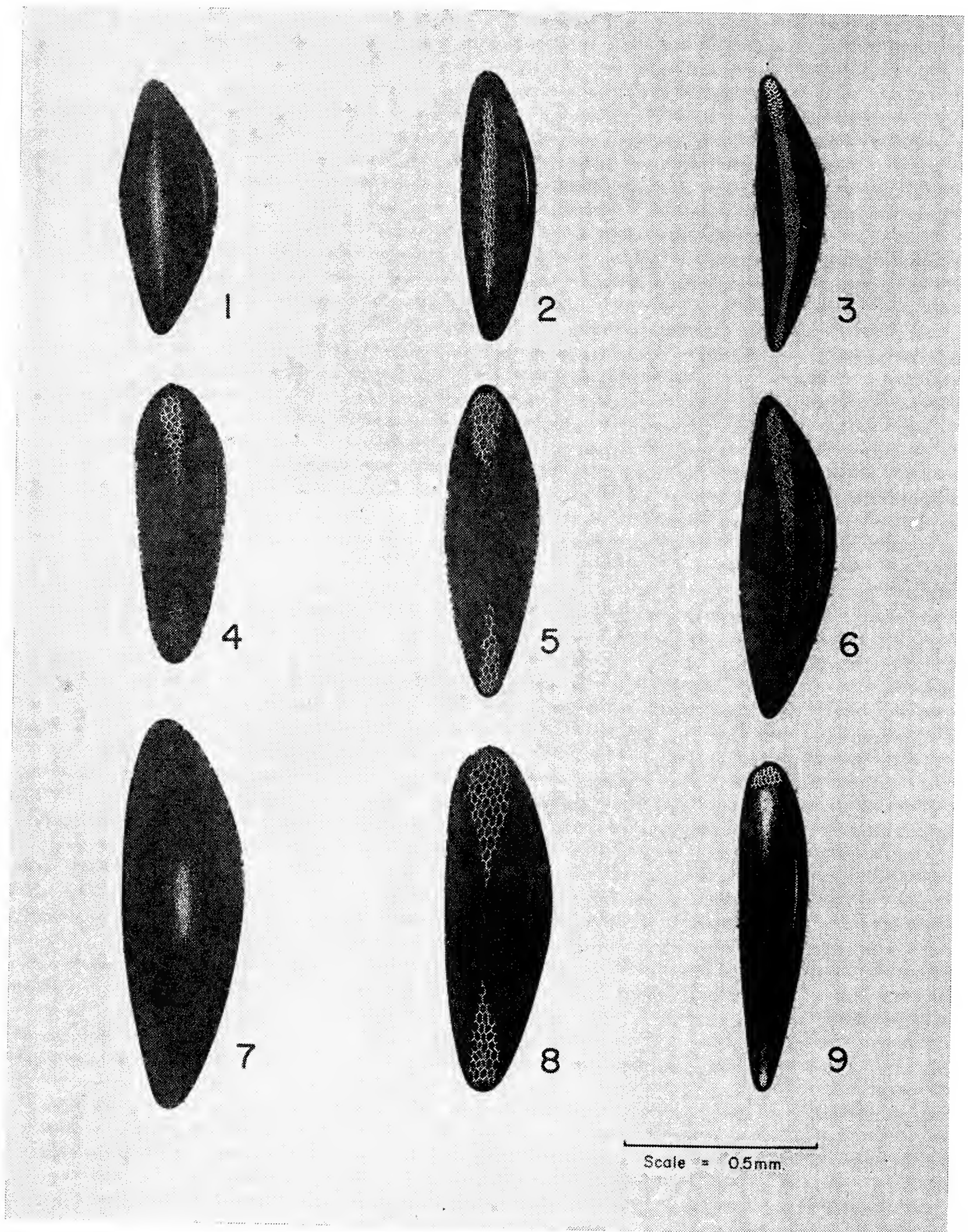


Plate I. Lateral aspect of eggs of species of Nearctic *Aedes*. Anterior pole is toward top; dorsal aspect toward left. Fig. 1. *A. sticticus*; Fig. 2. *A. vexans*; Fig. 3. *A. dupreei*; Fig. 4. *A. thibaulti*; Fig. 5. *A. canadensis*; Fig. 6. *A. sollicitans*; Fig. 7. *A. hexodontus*; Fig. 8. *A. stimulans*; Fig. 9. *A. cinereus*.



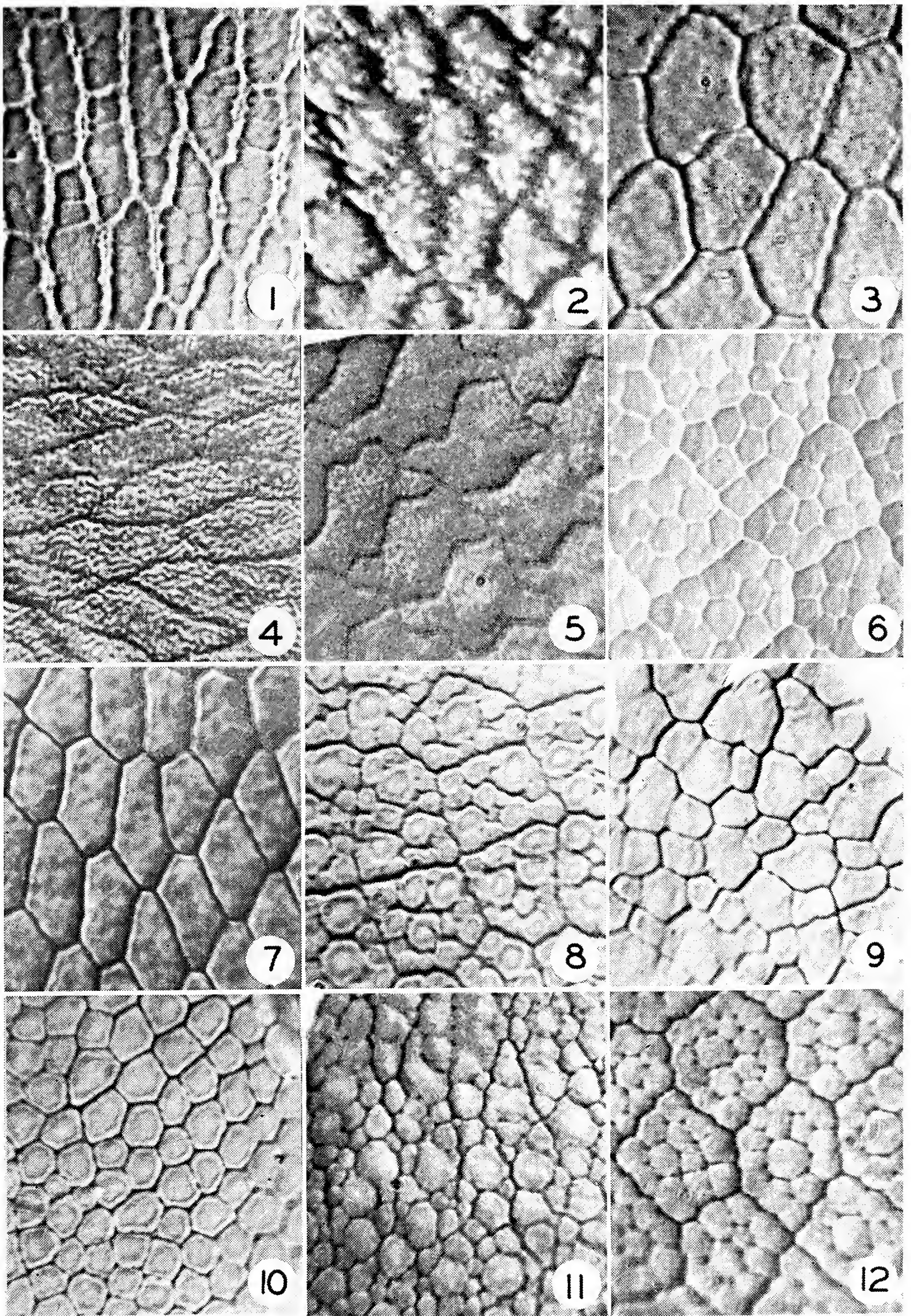


Plate II. Chorionic detail of eggs of species of Nearctic *Aedes*. Photographs are of bleached fragments as viewed through a phase-contrast microscope. Anterior pole is toward top. Fig. 1. *A. vexans*; Fig. 2. *A. atropalpus*; Fig. 3. *A. varipalpus*; Fig. 4. *A. aegypti*; Fig. 5. *A. sollicitans*; Fig. 6. *A. excrucians*; Fig. 7. *A. fitchii*; Fig. 8. *A. dorsalis*; Fig. 9. *A. dupreei*; Fig. 10. *A. communis*; Fig. 11. *A. implicatus*; Fig. 12. *A. thibaulti*.



Eggs of Nearctic *Aedes* are readily separable into smaller categories. They may be separated from the *Psorophora* by (1) absence of spiculose exochorion and (2) differences in curvature of the dorsal and ventral surfaces. Characteristics indicative of specific differences have been found for nearly all of the genus found in North America. The principal differences are in (1) size, (2) color and (3) sculpturing on the chorion.

For the most part eggs of species found in any one area are sufficiently distinctive that they may be separated readily with magnifications of about 100X. Size and shape may be of diagnostic value as is shown in Plate I. Some eggs are as short as  $440\mu$  and others are over a millimeter. In shape species vary from variously fusiform through ovoid to obovoid. Color may be brown, bronze, blue-black or satiny black according to species. Sculpturing of the chorion is particularly helpful. Some eggs have a uniform, clearly visible net covering their surfaces while the surfaces of others may appear lightly rugose, shagreened, cratered or nearly smooth. Characteristic differences in sculpturing may be evident between dorsal and ventral portions or between polar and equatorial surfaces. Lastly the cells comprising the chorionic reticulation differ according to species. Plate II illustrates a few examples of the numerous characteristic variations in shapes, walls and discs of these cells.

An analysis of specific characteristics and affinities based on external structures indicates that eggs of *Aedes* and *Psorophora* fall into natural categories similar to the arrangement of groups in the phylogenetic system of Edwards (1932) which is based largely on characteristics of adults. Therefore, external structures of eggs appear indicative of phylogenetic relationships among aedine mosquitoes. However, classification of species by means of eggs indicates certain exceptions to the system used by Edwards and to the validity of certain groups of species which are in current use. These exceptions give evidence for possible realignment of some categories. In some cases, the occurrence of discontinuous geographical variation among eggs from apparently similar parents collected in different localities appears to indicate subspeciation or may even demonstrate distinct species which have not previously been recognized.

Eggs provide a means for recognizing female mosquitoes. Most field collections consist of adult females which are often inadequate for identification yet may contain gravid specimens from which eggs may be removed. Eggs from this source or even those dissected from gravid museum specimens may be used for specific identification. Rubbed females or specimens belonging to a species complex with morphologically similar adults may, in many instances, be identified by characters found on eggs.

Eggs provide a useful source of information for investigators concerned with the bionomics of aedine mosquitoes. Since aedine eggs are restricted in their local distribution, are immobile and are present throughout most of the year, this stage of the life cycle appears to be most available to survey operations. Surveys based on distribution and abundance of eggs provide data which allow prediction of future populations of adult mosquitoes. Data on specific oviposition sites in the field also give information on the behavior and oviposition habits of adult females.

Sanitarians can use information obtained from eggs of floodwater mosquitoes in planning abatement operations. Surveys for eggs, supplemented by observations on the distribution of larvae and adults, allow formulation of a comprehensive picture of the distribution and abundance of pest species. These surveys may be conducted during the fall and winter months when regulatory operations are inactive. Such egg surveys are currently being used with considerable success by certain municipal mosquito abatement districts and other regulatory organizations.

The value of abatement measures directed against eggs and ovipositing females should be considered. The distribution of insecticides as a prehatching treatment against overwintering eggs is now a standard practice in many areas. Such treatments could be conducted in a more economical fashion if accurate data on specific oviposition sites were available. Permanent control measures requiring elimination of specific oviposition sites without further alteration of the environment offer significant possibilities.

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### DISCUSSION

R. LEVI-CASTILLO. Have you used the usual techniques for female oviposition in the laboratory? In Ecuador we have used the test tube with a piece of wet filter paper. We hit the tube on the palm of the hand and after 24 hours obtain oviposition.

G. B. CRAIG, JR. By retaining the blooded female in a small tube with moist cellulose cotton, we usually get oviposition by 60 to 80% of the specimens. This can be facilitated by the addition of a dried raisin or by giving a second blood meal.



# Der frühjährliche Temperaturgang in Berliner *Aedes*-Brutgewässern (Diptera: Culicidae). (Beiträge zur Kenntnis der *Culicidae* IV)

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## ZUSAMMENFASSUNG

In Zusammenhang mit der Stechmückenbekämpfung in einem Erlenbruch bei Berlin wurden Messungen der Wassertemperatur in *Aedes*-Brutgewässern während der frühjährlichen Entwicklung der Präimaginal-Stadien ausgeführt. Gleichzeitig wurde in möglichst regelmässigen Abständen während der ganzen Zeit der Anteil der einzelnen Larven und Puppenstadien der drei hauptsächlich vertretenen Arten—*Aedes cantans* Mg., *A. punctor* Kirby und *A. cinereus* Mg.—ermittelt. Die erhaltenen Werte, in Tabellenform zusammengefasst, sollen als Arbeitsgrundlage dienen, vor allem zur Untersuchung der Zusammenhänge zwischen dem Ablauf der Larven- sowie der Puppenentwicklung und dem frühjährlichen Temperaturgang in den kleinen, als Brutgewässer bevorzugten temporären Tümpeln. Einige Hinweise in dieser Richtung werden gegeben.

Den eigentlichen Anlass zu den vorstehenden Untersuchungen gaben Schwierigkeiten bei den Zuchten der hiesigen Frühjahrs-*Aedes*, als deren häufigste Vertreter *Aedes cantans* Mg., *A. punctor* Kirby, *A. cinereus* Mg., *A. excrucians* Walker und *A. communis* De Geer genannt seien. Bei der Suche nach den Gründen konnte nicht übersehen werden, dass der Wassertemperatur eine grosse Bedeutung in dieser Hinsicht beizumessen war. Während nun in der erreichbaren Literatur entsprechende Messwerte im Freiland für die in perennierenden Gewässern sich entwickelnden heimischen *Culex*- und *Anopheles*-Arten vorlagen, fehlten sie weitgehend für die in temporären "Tümpeln" lebenden *Aedes*-Arten. Dieses gilt im besonderen für die Waldformen; für die in unbeschatteten Gelände sich entwickelnden, oder als "Gewitterbrüter" bekannten Wiesen-*Aedes* konnten einige Temperaturdaten in der Literatur gefunden werden, wenn auch nur Einzelwerte, aber keine Reihenmessungen. Für Lappland (National-Park von Abisko) mit seinen völlig anders gearteten klimatischen Bedingungen liegen solche letzteren vor (Thienemann, 1939), die jedoch für Mitteleuropa keine Bedeutung haben. So entstand der Entschluss, die fehlenden Unterlagen—zunächst für die hiesigen Wald-*Aedes*—selbst zu schaffen. Die Ergebnisse mögen als bescheidener Beitrag zur Kenntnis der Stechmücken sowie als Arbeitsgrundlage im folgenden bekanntgegeben werden.

Für die Untersuchungen bot sich ein ergiebiger *Aedes*-Brutplatz im "Grunewald", einem im Südwesten Berlins noch innerhalb der Stadtgrenzen gelegenen, mehr oder weniger ursprünglichen Waldgelände. Der Ort war vom Institut aus leicht mit dem Fahrrad zu erreichen, so dass die notwendige Überwachung gewährleistet war. An eine Temperaturmessung mit laufender automatischer Registrierung konnte in Anbetracht der damaligen Knappheit an Mitteln nicht gedacht werden. Der *Aedes*-Brutplatz befand sich in einem als "Langes Luch" bezeichneten Erlenbruch, welches im Zuge der Grunewaldseenrinne zwischen dem Grunewaldsee und dem Riemeister-Fenn gelegen war. Der Grundwasserstand war damals in Berlin als Folge der Kriegs- und Nachkriegsverhältnisse sehr hoch, so dass das Erlenbruch im Frühjahr weithin überschwemmt war (Abb. 1). Entsprechend dem Bodenrelief gab es stellenweise keine zusammenhängende Wasserfläche, sondern trockene Inseln und mehr oder weniger tiefe Tümpel von verschiedener Grösse. Das Gelände war wegen der ursächlich mit dem abnorm hohen Wasserstand zusammenhängenden Mückenplage vor zwei Jahren Gegenstand einer Behandlung mit Larviziden gewesen. Dennoch fand sich bereits wieder reichlich *Aedes*-Brut, so dass die geplanten Untersuchungen möglich waren. Inzwischen ist übrigens der Grundwassertand in Berlin wieder auf den normalen Stand gesunken. Ausserdem hat man ihn im Bereich des "Langen Luches" durch Führung eines Kanals noch tiefer gelegt, so dass die Zahl der *Aedes*-Brutplätze stark vermindert ist. Mit einer gewissen Wehmut—für den Spaziergänger unverständlich—betrachtet der Culicidologe, zumal wegen der gegenwärtigen politischen Insellage Berlins, die nun schon dokumentarischen Fotos und erinnert sich der verlorengegangenen Untersuchungsmöglichkeiten in diesem "Mücken-Paradies".



Abb. 1. Das "Lange Luch" bei Berlin, in dem die Messungen durchgeführt wurden. Abb. 2. Die Messstelle mit den an Schwimmflößen befestigten Maximum-Minimum-Thermometern.

Das "Lange Luch" ist ein langgestrecktes Erlenbruchgebiet vom *Rubus idaeus*-Typ (Hueck, 1942). Der zum Teil recht dichte Unterwuchs besteht, vor allem im Bereich unserer Messstelle, aus Faulbaum (*Rhamnus frangula*) und Himbeere (*Rubus idaeus*); Binsen, weiche Riedgräser, Farne und Moose, darunter reichlich Torfmoos (*Sphagnum*), vervollständigen das Bild. An den Rändern des Luches wuchern Brombeeren. Für unsere Untersuchungen wählten wir einen mehrere qm grossen Tümpel in der Mitte des Geländes. Die Wassertiefe betrug anfangs ungefähr 40 cm. Sie verringerte sich während der Beobachtungszeit durch Sonneneinwirkung auf 25 cm, starke Niederschläge liessen sie jedoch vorübergehend wieder bis auf 35 cm ansteigen. Wir begannen mit den Messungen am 15.3.1950. [Wegen der vorangegangenen kalten Nächte wies der Tümpel noch eine Eisschicht von etwa 2 mm Stärke auf. Sie wurde zur Messung der Wassertemperatur beseitigt. Das gleiche geschah mit der in der folgenden Nacht entstandenen Eisschicht von 4 mm Stärke. Danach fand keine Eisbildung mehr statt.] Die Messung der Wassertemperaturen erfolgte mit den üblichen Maximum-Minimum-Thermometern. Um sie an der Wasserober-



TABELLE I— Anteil der Larvenstadien und des Puppenstadiums von *Aedes cantans* Mg.\* bei Probefängen im Messtümpel in Prozent.

Datum (1950)	I. Stad.	II. Stad.	III. Stad.	IV. Stad.	Puppen- stad.
15.3	100				
18.3	90	10			
21.3	70	30			
22.3	60	40			
23.3	40	60			
27.3		78	22		
29.3		50	50		
3.4		25	75		
8.4		8	80	12	
13.4			20	80	
18.4			8	92	
21.4				100	
25.4				50	50
1.5				30	70
2.5				10	90
3.5					100

\*Bis einschliesslich 27.3.1950 vermischt mit *A. punctor*. Vergl. S. 862.

TABELLE II— Anteil der Larvenstadien und des Puppenstadiums von *Aedes punctor* Kirby\* bei Probefängen im Messtümpel in Prozent.

Datum (1950)	I. Stad.	II. Stad.	III. Stad	IV. Stad.	Puppen- stad.
29.3	—	—	100		
3.4			100		
8.4			51	49	
13.4			24	76	
18.4				100	
21.4				30	70
25.4				10	90
1.5					100

\*I. und II. Stadium siehe unter *A. cantans* Mg. (Tab. I).

fläche, dem bevorzugten Aufenthaltsort der Larven, zu halten, wurden sie durch kleine Holzflösse schwimmbar gemacht (Abb. 2). Da es uns darauf ankam, die eigentliche Wasser-

TABELLE III—Anteil der Larvenstadien und des Puppenstadiums von *Aedes cinereus* Mg. bei Probefängen im Messtümpel in Prozent.

Datum (1950)	I. Stad.	II. Stad.	III. Stad.	IV. Stad.	Puppen- stad.
18.4	—	100			
21.4		50	50		
25.4			20	80	
1.5				50	50
2.5				30	70
3.5				10	90
4.5					100

temperatur zu messen und die Einwirkung der Sonneneinstrahlung zu vermeiden, wurden die Thermometer mit Riedgras abgedeckt.

Da sich morgens häufig keine Mückenlarven an der Wasseroberfläche befanden, sie sich vielmehr alle am Grunde aufhielten, legten wir dorthin ab 22.3.1950 ein weiteres Thermometerpaar, das von diesem Zeitpunkt an zusammen mit dem Oberflächenpaar täglich gegen 9 Uhr abgelesen wurde. Ausserdem wurde die Augenblickstemperatur des Wassers an der Oberfläche und auf dem Grunde des Tümpels gemessen. Um ferner die Schwankungen der Lufttemperatur nahe der Messstelle kennenzulernen, wurde vom 30. März 1950 an dort ein 3. Thermometerpaar angebracht und zur gleichen Zeit (gegen 9 Uhr) täglich regelmässig abgelesen. Mit einem Schleuderthermometer wurde endlich noch die augenblicklich herrschende Lufttemperatur bestimmt. Zur Charakteristik des Brutgewässers sei gesagt, dass nach den Befunden des wassertechnischen Laboratoriums unseres Institutes eine Wasserprobe als klar und durch gelöste Huminstoffe bräunlich gefärbt bezeichnet wurde. Die Reaktion gegen Lackmus war sauer, die Wasserstoffionenkonzentration (pH) 5,6. Der Geruch wurde als "schwach erdig" bezeichnet. Wohl mit bedingt durch den hohen Anteil an gelösten Huminsäuren war der Kaliumpermanganatverbrauch hoch, er betrug 732 mg/1. Die Härte der Wasserprobe muss als sehr gering bezeichnet werden, sie wurde zu 2,2 Deutsche Härtegrade ermittelt.

Die Zusammensetzung der Larvenpopulation entsprach ganz der in hiesigen Erlenbrüchern mit hochmoorartigem Einschlag üblichen. Die Hauptvertreter waren *Aedes cantans*, *A. punctor* (allgemein als tyrphophil angesehen) und *A. cinereus*, dazu kamen einige wenige *A. excrucians*. Um die Larvenentwicklung in Beziehung zu den Temperaturmessungen setzen zu können, wurden in möglichst regelmässigen Abständen, beginnend mit dem 15. März, Larvenproben entnommen und im Laboratorium nach Zugehörigkeit zu Art und Stadium untersucht. Hierbei leistete das vorzügliche Werk von Marshall "British Mosquitoes" treffliche Dienste. Wir müssen allerdings gestehen, dass die—übrigens schwierige—artliche Separierung von *A. cantans* und *A. punctor* zunächst nicht erfolgte. Wir hielten die Population anfangs für homogen, und erst allmählich kamen wir dahinter, dass 2 Arten vertreten waren. Vom 29.3. an trennten wir die beiden Spezies korrekt. *A. cinereus*, der erst später kinzukam, wurde gleich von Anfang an leicht erkannt. *A. excrucians* wurde wegen seines nur vereinzelt Vorkommens ausser Betracht gelassen. Was die Mengenverhältnisse der Arten in den Proben anbelangt, so dominierte *A. cantans* meist—zum Teil erheblich—über *A. punctor*; *A. cinereus* gelangte zunächst nur selten zur Beobachtung. Seine Mengenentfaltung entsprach jedoch später in etwa der der beiden anderen Arten. Zum Vergleich mit den Freilandbedingungen wurden Larven im Laboratorium unter nahezu konstanten Bedingungen (Raum-Temperatur  $\pm 20^{\circ}\text{C}$ ) gehalten. Die am 15.3. in das Labor genommenen Erstlarven erreichten hier teilweise bereits am 21.3. das 4. Stadium; am 25.3. war die erste Puppe zu sehen und bereits am 27.3. schlüpfte die erste Mücke, ein *Aedes cantans* ♂, am 29.3. dann ein *A. cantans* ♀, am 30.3. ein *A. punctor* ♀, am 31.3. schliesslich das erste *A. punctor* Männchen. Im günstigsten Falle

TABELLE IV.

Datum	Extremtemperatur °C. im Tümpel*			TpD = Mi + Ma 2 °C.	Lufttemperatur °C.*			TpD = Mi + Ma 2 °C.	Sonnen- schein- dauer i. Std.**	Nieder- schlags- menge in mm.**
	Max.	Min.	Ampli- tude		Max.	Min.	Ampli- tude			
15.3.	nur Augenblickstemp.								4,0	—
16.3.	6,8	0,5	6,3	3,7					10,0	—
17.3.	8,0	1,0	7,0	4,5	9,0				8,0	—
18.3.	11,0	3,5	7,5	7,3	11,0				10,0	2,0
19.3.	13,8	4,3	9,5	9,1	14,0				5,0	—
20.3.	12,3	4,8	7,5	8,6	12,0				7,5	—
21.3.	11,8	5,0	6,8	8,4	9,5				5,5	—
22.3.	11,0	5,5	5,5	8,3	10,5				8,5	—
23.3.	13,3	6,5	6,8	9,9	12,5				7,5	—
24.3. o†	13,0	4,0	9,0	8,5						
u	9,0	5,5	3,5	7,3	11,0				8,0	—
25.3. o	12,5	5,5	7,0	9,0						
u	9,0	5,3	3,7	7,2	7,5				0,1	2,8
26.3. o	10,5	6,3	4,2	8,4						
u	9,0	6,0	3,0	7,5	9,5				9,5	—
27.3. o	13,0	3,5	9,5	8,3						
u	11,5	5,0	6,5	8,3	8,0				12,0	—
28.3. o	11,5	3,5	8,0	7,5						
u	8,0	4,0	4,0	6,0	4,0				4,0	—
29.3. o	9,0	2,5	6,5	5,8						
u	8,3	4,0	4,3	6,2	5,5				6,0	—
30.3. o	9,0	3,0	6,0	6,0						
u	7,3	3,8	3,5	5,6	7,0				3,5	—
31.3. o	9,0	3,0	6,0	6,0						
u	7,5	4,5	3,0	6,0	10,0	-2,0	12,0	4,0	7,5	0,1
1.4. o	10,0	4,0	6,0	7,0						
u	7,5	4,5	3,0	6,0	11,5	4,5	7,0	8,0	—	—
2.4. o	8,0	5,0	3,0	6,5						
u	7,0	5,0	2,0	6,0	10,0	5,0	5,0	7,5	—	1,0
3.4. o	9,5	3,5	6,0	6,5						
u	7,5	4,5	3,0	6,0	12,0	-1,5	13,5	5,3	6,4	2,8
4.4. o	10,0	3,5	6,5	6,8						
u	8,5	4,0	4,5	6,3	16,0	-1,5	17,5	7,3	5,8	—
5.4. o	10,5	3,0	7,5	6,8						
u	10,0	3,5	6,5	6,8	13,0	-0,5	13,5	6,3	2,9	0,5
6.4. o	10,0	4,5	5,5	7,3						
u	10,0	4,8	5,2	7,4	12,5	1,5	11,0	7,0	2,5	11,7
7.4. o	9,0	3,5	5,5	6,3						
u	10,0	4,0	6,0	7,0	11,5	-2,0	13,5	4,8	10,6	1,9
8.4. o	12,0	2,5	9,5	7,3						
u	11,3	4,5	6,8	7,9	22,5	-3,3	25,8	9,6	10,4	—
9.4. o	13,5	4,0	9,5	8,8						
u	10,5	4,5	6,0	7,5	22,0	7,0	15,0	14,5	4,0	—

TABELLE IV—Cont'd.

Datum	Extremtemperatur °C. im Tümpel*				Lufttemperatur °C.*				Sonnen- schein- dauer i. Std.**	Nieder- schlags- menge in mm.**
	Max.	Min.	Ampli- tude	TpD = Mi+Ma 2 °C.	Max.	Min.	Ampli- tude	TpD = Mi+Ma 2 °C.		
10.4.o	13,3	5,5	7,8	9,4						
u	11,0	6,0	5,0	8,5	19,0	4,5	14,5	11,8	7,7	0,4
11.4.o	12,5	4,5	8,0	8,5						
u	8,8	5,0	3,8	6,9	13,5	2,0	11,5	7,8	6,2	3,9
12.4.o	10,5	4,0	6,5	7,3						
u	10,0	4,5	5,5	7,3	13,0	-1,0	14,0	6,0	5,0	1,0
13.4.o	13,0	5,0	8,0	9,0						
u	10,0	5,0	5,0	7,5	17,0	±0	17,0	8,5	4,7	4,0
14.4.o	12,0	4,3	7,7	8,2						
u	10,0	4,8	5,2	7,4	13,0	-2,0	15,0	5,5	8,1	12,2
15.4.o	12,5	5,0	7,5	8,8						
u	11,0	4,8	6,2	7,9	15,0	1,5	13,5	8,3	—	—
16.4.	keine Ablesung									24,6
17.4.o	7,5	3,3	4,2	5,4						
u	8,3	5,5	2,8	6,9	7,0	4,0	3,0	5,5	5,2	—
18.4.o	13,5	4,0	9,5	8,8						
u	11,5	4,8	6,7	8,2	15,0	-2,5	17,5	6,3	10,2	—
19.4.o	15,0	5,5	9,5	10,3						
u	14,0	4,8	9,2	9,4	23,0	0,5	22,5	11,8	12,8	—
20.4.o	17,5	7,5	10,0	12,5						
u	16,5	8,0	8,5	12,3	25,0	4,0	21,0	14,5	0,6	—
21.4.o	13,5	8,5	5,0	11,0						
u	12,8	8,3	4,5	10,6	17,0	8,0	9,0	12,5	—	1,6
22.4.o	13,3	9,5	3,8	11,4						
u	13,0	9,5	3,5	11,3	14,0	9,0	5,0	11,5	—	3,0
23.4.o	13,0	9,5	3,5	11,3						
u	12,5	10,0	2,5	11,3	12,0	8,0	4,0	10,0	—	—
24.4.o	13,0	8,5	4,5	10,8						
u	11,5	9,0	2,5	10,3	15,0	6,0	9,0	10,5	1,2	7,0
25.4.o	14,5	8,0	6,5	11,3						
u	12,5	8,0	4,5	10,3	15,5	4,5	11,0	10,0	4,6	0,1
26.4.o	13,0	5,5	7,5	9,3						
u	11,0	6,0	5,0	8,5	15,5	5,0	10,5	10,3	12,5	0,2
27.4.o	16,5	6,0	10,5	11,3						
u	14,5	6,0	8,5	10,3	22,0	2,5	19,5	12,3	5,9	—
28.4.o	13,5	6,0	7,5	9,8						
u	10,8	6,5	4,3	8,7	15,5	4,5	11,0	10,0	3,2	0,4
29.4.o	11,0	5,0	6,0	8,0						
u	10,0	5,5	4,5	7,8	12,5	0,5	12,0	6,5	9,3	4,6
30.4.o	12,5	4,5	8,0	8,5						
u	11,0	4,5	6,5	7,8	19,0	-4,0	23,0	7,5	10,7	1,0



TABELLE IV—Cont'd.

Datum	Extremtemperatur °C. im Tümpel*			TpD = Mi+Ma	Lufttemperatur °C.*			TpD = Mi+Ma	Sonnen- schein- dauer i. Std.**	Nieder- schlags- menge in mm.**
	Max.	Min.	Ampli- tude	2 °C.	Max.	Min.	Ampli- tude	2 °C.		
1.5.o	17,5	7,5	10,0	12,5	21,5	3,0	18,5	12,3	12,8	—
u	12,0	7,0	5,0	9,5						
2.5.o	19,5	10,0	9,5	14,8	31,0	7,5	23,5	19,3	12,6	—
u	12,5	9,5	3,0	11,0						
3.5.o	18,5	12,0	6,5	15,3	33,0	11,5	21,5	22,3	0,2	0,2
u	19,0	11,5	7,5	15,3						
4.5.o	14,0	10,5	3,5	12,3	17,5	8,0	9,5	12,8	3,7	1,1
u	14,0	10,3	3,7	12,2						
5.5.o	14,0	10,0	4,0	12,0	22,0	6,0	16,0	14,0	1,7	—
u	14,5	10,0	4,5	12,3						
6.5.o	15,5	11,8	3,7	13,7	17,0	11,0	6,0	14,0	—	12,3
u	15,0	10,5	4,5	12,8						

\*Die zur Zeit der Ablesung gemessenen Augenblickswerte in Wasser und Luft sind zwecks Raumersparnis weggelassen. Anordnung in Anlehnung an Thienemann, 1939, um Vergleiche zu erleichtern.

\*\*Die Werte verdanken wir dem Meteorologischen Institut der Freien Universität Berlin.

†o = Messungen an der Oberfläche; u = Messungen am Grund.

dauerte die Entwicklung im Labor also nur 12 bis 13 Tage. Ausserdem zeigte sich hierbei, dass beide Arten unter den Erstlarven des 15.3. vertreten gewesen waren.

Die Tabellen I-III zeigen für die 3 *Aedes*-Arten die Anteile der Larvenstadien I-IV sowie des Puppenstadiums an den über die ganze Beobachtungszeit verteilten Probeentnahmen im Freiland.

Die Tabelle IV enthält nun die Ergebnisse der Temperaturmessungen in dem Beobachtungstümpel während der Zeit vom 15.3.1950 bis zum 6.5.1950. Die Bedeutung der einzelnen Spalten ist aus den am Kopf gemachten Angaben ersichtlich.

Es war zunächst beabsichtigt, die in der Tabelle IV niedergelegten Messwerte zu den einzelnen Witterungsfaktoren in Vergleich zu setzen. Damit hätte die vorliegende Arbeit dann aber den gebotenen Umfang überschritten. So soll davon hier Abstand genommen werden, ebenso von der geplanten Feststellung der für die Entwicklung notwendigen Temperatursumme und des Entwicklungsnullpunktes. Weitere vorgesehene Messungen, vergleichsweise auch für die in unbeschattetem Gelände brütenden sog. "Wiesen-*Aedes*", dürften eine willkommene Grundlagenverbreiterung zu den dafür notwendigen Berechnungen geben.

Auf einige wenige Ergebnisse der Messungen in Bezug auf das Larvenleben sei hingewiesen. Die Erstlarven von *Aedes cantans* und *A. punctor* entwickelten sich in etwa 50 Tagen zur Mücke. Die tiefste während dieser Periode gemessene Wassertemperatur im Bruttümpel betrug wenig über 0° (0,5° am 15.—16.3.1950), die höchste nahezu +20° (19,5° am 2.5.1950). Die Neigung der Larven, vor allem in den kalten Morgenstunden, die Oberfläche des Brutgewässers zu meiden und lieber am Grunde des Tümpels zu weilen, konnte durch die Messungen, wie angenommen, als positive Thermotaxis bestätigt werden. Die tiefsten Werte betrugen z.B. oben/unten: Am 24.3.1950: 4,0°/5,5°; am 27.3.: 3,5°/5,0°; am 8.4.: 2,5°/4,5° und am 17.4.: 3,2°/5,5°. Am Grunde waren also gelegentlich bis zu 2° höhere Temperaturen vorhanden als an der Oberfläche.

Diese wenigen Hinweise mögen hier genügen. Der interessierte Leser wird ohne Schwierigkeiten weitere Zusammenhänge zwischen den Messunterlagen und den Lebenserscheinungen der *Aedes*-Larven während ihrer Entwicklung auffinden können.

## LITERATURVERZEICHNIS

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- Thienemann, A. 1939. Frostboden und Sonnenstrahlung als limnologische Faktoren. *Arch. Hydrobiol.* XXXIV: 306–345.

## DISCUSSION

A. S. MONTSCHADSKY. Haben sie die Intensität der Sonnenradien parallel gemessen? Wenn nicht, dann sind ihre Angaben über die Abhängigkeit der Geschwindigkeit der Entwicklung nicht vollständig.

E. W. KIRCHBERG. Die Intensität der Sonneneinstrahlung wurde nicht gemessen. Es wurde angenommen, dass die Larven als poikilotherme Tiere von der an der Wasseroberfläche gemessenen Temperatur in ihrer Entwicklungsgeschwindigkeit gesteuert werden.

R. LEVI-CASTILLO. Ich wollte wissen wie lange Zeit haben sie gearbeitet mit diese systematischen Messungen und wie lange haben sie ihre Messungen gemacht?

E. W. KIRCHBERG. Die Untersuchungen wurden im Frühjahr 1950 durchgeführt und nicht wiederholt.

C. F. A. BRUIJNING. Gibt es nur eine Generation bevor die Tümpeln austrocknen?

E. W. KIRCHBERG. Im allgemeinen ja. Gelegentlich folgt im Sommer eine zweite unvollkommene Generation.

# Provisional List of the Culicidae, Simuliidae, *Phlebotomus* and *Culicoides* of Ecuador

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## ABSTRACT

*A climatological description and provisional lists of the Culicidae, Culicoides, Phlebotomus and Simuliidae of Ecuador are given as studied by the "Ecuadorian Center for Entomological Research".*

## INTRODUCTION

The study of the Culicidae, Simuliidae, *Phlebotomus*, and *Culicoides* of Ecuador is of great importance because some of these insects readily become the vectors of tropical diseases. The "Ecuadorian Center for Entomological Research" was organized in 1951 as a non-profit private organization to study the insect fauna of the whole Ecuadorian territory. The study of the Culicidae has been the main work of the "Center" and the author has studied the material now in the collection and the lists here given have been brought up-to-date. The study of Simuliidae, *Phlebotomus*, and *Culicoides* has been conducted by some of the Ecuadorian entomologists, working part time in the subject, and also foreign entomologists such as Dr. Luis Vargas and Mr. Alfonso Diaz Najera, who study the Simuliidae and Dr. Willis W. Wirth who determined the Ecuadorian *Culicoides*, because we have no specialists in these groups.

The research work now being conducted at the Center is as follows:

1. Field research on the geographical distribution, ecology, biology, physiology, systematics and taxonomics, ethology, high altitude studies, faunal extension of species, geographical and environmental variation, resistance to insecticides, etc.
2. Laboratory work at the field station at "Cuatro Hermanitos Experimental Farm", and the private laboratory and library of the institution located at the author's home.
3. Material is sent to specialists in the many fields in which there are no specialists in Ecuador and consultation is established with specialists of the highest standing in their fields of research.
4. The journal "Revista Ecuatoriana de Entomologia y Parasitologia" is published by the institution with the help of the "Nucleo del Guayas de la Casa de la Cultura Ecuatoriana" in whose presses it is now being processed. Since 1953, only Volumes I and II have been published to date.

## BIOCLIMATOLOGY OF ECUADOR

It is necessary to understand the many biotopic changes observed in Ecuador that determine speciation of the Diptera in accordance with the environmental changes observed in their individual biotopes. The dispersion of species is limited by climatic changes such as atmospheric pressure, environmental humidity, changes in temperature, such as extreme cold or heat (this last factor for high altitude biotopes of high altitude species when brought to sea level and vice versa), altitude of mountains, winds, vegetation, barren lands, desert, etc. The bioclimatology of Ecuador is so complex that species are confined by small climatic changes to definite regions, and their dispersion is so limited that one is unable to find them in a biotope different from the one in which they usually live, probably because of climatization and adaptation to environmental conditions in one place through hundreds and probably thousands of years. Take for example high altitude mosquitoes 2,500 to 4,200 meters above sea level. They are adapted to low atmospheric pressure, cold, low oxygen content of atmosphere, cold winds, etc., factors predominating in their habitat, where they are limited to tree-top Bromeliaceae and some to small mountain pools, under most trying conditions. These mosquitoes even though of a poikilothermic nature are unable, because of their environmental adaptation, to withstand higher temperatures, higher oxygen content, and higher atmospheric pressure than are found in the lowlands of the Coastal Plain. On the other hand, for the same reasons, coastal mosquitoes readily die if transported to high altitudes.

The Cordillera of Los Andes, the Pacific Ocean, and other ecological limits and also the variety of climatic conditions encountered serve to maintain the distribution of species and limit their dispersion to sections of definite ecologic populations. Actual practice enables an entomologist familiar with the ecologic conditions in Ecuador to know immediately, at least in the Culicidae, from what region a lot of specimens came. For example, *Wyeomyia* spp. are found only in the Coastal Plain and the Amazon Basin, but never at an altitude above 1,500 meters; *Haemagogus* are found in Ecuador only up to 2,000 meters above sea level, on the slopes of the Andes, but never in the warm highland valleys, where species are very limited in scope.

The Ecuadorian Center for Entomological Research has been the first institution to organize the study of the bioclimatic conditions of the insect fauna of Ecuador. These observations are too extensive for presentation before this Congress, but will be published in the near future in a series of monographic papers now in preparation by their authors.

Climate is one of the most important of the environmental biophysics factors that determine the living conditions of insects which, although of a poikilothermic nature, are readily influenced by minute variations in their microclimatic environment. Thus, in Ecuador insects are limited in their dispersion by climate, atmospheric pressure, humidity, high altitude, vegetation, presence or absence of rains, etc. There is a great variety in the climate of Ecuador. In a few hours one is able to ride from the tropical into the cold temperate and polar climates of the "Paramos" where one finds snow and ice. Atmospheric pressure changes also very abruptly from 760 mm in the Western Plain near the seashore to 547.3 mm in the Highlands. Climatic changes are very extensive and in one short distance one can find more than 20 different climatic changes due to outstanding geographical accidents. This has made Ecuador a country where it is usual to find the unusual, and it is a paradise for entomologists, still virgin because we have just started entomological studies on a limited scale. But to be able to do anything worthwhile, any person interested in collecting in my country needs to consider staying a long time for seasonal variations and regional observations, involving expenses and good means of transport. We now have beautiful roads covering all the provinces and, as time goes by, new highways are being improved and built to places that not long ago were isolated from all contact with civilization. One is now able to ride on good roads not only in the Coastal Plain but also in the Amazon basin.

We have been able to explore to date only 25% of our national territory in 5 years of continuous effort and still there is much to be done; a lifetime is not enough to complete all the studies that still have to be done. On the western slopes of the Andes there are large jungles still unexplored and in the Amazonic basin 99% of the territory is still to be explored and studied. On reaching a new location many unknown and unnamed geographical features are observed such as ravines, mountain streams, rivers and small orographic accidents, such as small hills and chains of mountains, etc., in places where no detailed map exists to date. Sometimes our teams must record these geographical features for faunistic ecological studies.

The main climatic influence in the country is the Andes Cordillera that divides the Republic of Ecuador into three geographical regions that are different in all respects:

1. *The Western Region (Coastal Plain)* is the extension running from the Western slopes of the Andes to the Pacific Ocean.
2. *The Highlands (Sierra or Interandean Region)* extends between the two chains of the Andes, comprising the warm valleys located into the hileae of the andean chain of mountains and orographic knots, that limit the dispersion of high altitude species of insects.
3. *Amazonic Region (Amazonic Plain or Hilea Amazonica)* extends from the eastern slopes of the Andes into the river system of the Amazon Plain, all covered with tropical rain-forest.

The *Cold Humboldt Current* is the main climatic influence of the Coastal Region. It usually has a medium temperature of 20°C., modifying the heat and humidity of the zone between the Islands of Jambeli and Cape Pasado, and producing a notable and marked climatic change because it comes from the South Pole as soon as the ice cap begins to melt. Thus the amount of cold water in the Humboldt Current attains its peak in August



through October. This cold current diminishes the atmospheric vapor tension and relative humidity, participating the subregion through which it passes of a dry and arid climate, as the cold current approaches the seashore. Temperatures in this subregion are between  $12^{\circ}$  and  $24^{\circ}\text{C}$ . in the dry season, and never rise above  $33^{\circ}\text{C}$ . in the rainy season, this being the maximum heat observed as the current diminishes its flow with the onset of the Antarctic winter. The same climate of this *Southern Subregion* is found from Tumbes in the south to Cape Pasado and then as it changes towards the west it goes through the Galapagos Islands. In this southern subregion we have to consider another important climatic modification. This is the region comprising Manglaralto, Guayas province, to El Aromo in Manabi province. This Sub-Subregion is influenced by the Colonche Mountains that line the seashore and rise from 300 to 500 meters above sea level. Although it has not such a high altitude in comparison with the Andean chain, yet its climatic influence is enormous on the zonal climate, because, during the dry season in the rest of the Southern Subregion, the mountain tops in this Sub-Subregion seem to serve as containing obstacles to the humidity created by the cold Humboldt Current and it rains during the coldest months, this is the only place in Ecuador where it rains all the year around in the Coastal Plain especially between June and November. This cold rain makes the temperature very low, especially at night, reaching  $8^{\circ}\text{C}$ . with a maximum of only  $23^{\circ}\text{C}$ ., a climate very much like the one observed in autumn in the Temperate Climates. Thus the fauna and flora is entirely different from the rest of the Southern Subregion, with a notable extension of limited rain-forests.

The *Northern Subregion* comprises the territory extending to the North of Cape Pasado and is characterized by its tropical climate; very humid and with enormous areas of rain-forest with minimum temperatures of  $24^{\circ}\text{C}$ . and maximum of  $35^{\circ}\text{C}$ . The climate of the Coastal Region depends on latitude and the influence of sea currents, and also gets cooler as it approaches the slopes of the Andes and as it recedes from the seashore. The Coastal Region is not a continuous plain, but is irrigated by many rivers and is very accented, orographically speaking, thus determining slight and great changes, depending on the vegetation and nearness to the Andean mountains. This is not found in any other country in South America. Because of its geographic situation in the center of the World at the Equator, from which takes its name, the Republic of Ecuador enables one to find all the climates of the world within short distance of one another.

The *Interandean Region* has many climatic variations because Andean Ecuador is not an alley, nor a limited wall-like structure to both sides due to the high Andean mountains. These ramify, forming two chains with a middle highland, limited by knots into small warm valleys that lie deep between the limiting mountains, with abundant highland rivers and streams produced by melting snows on the high peaks of the Andes and located at altitudes between 1,000 and 4,500 meters above sea level. Of great interest in the bioclimatic study of Ecuador are the great variations in atmospheric pressure. Wolf, a German geographer who studied the climates and climatic changes of Ecuador all his lifetime observed in 1896 that as soon as one ascends, the pressure descends and the temperature gets lower. He also observed that for each 200 meters of altitude the temperature fell  $1^{\circ}\text{C}$ . Thus, in Guayaquil, located at sea level, if he recorded a temperature of  $26^{\circ}\text{C}$ ., the temperature at the same time at 1,200 meters was only  $20^{\circ}\text{C}$ . and at 5,000 meters  $0^{\circ}\text{C}$ ., the temperature of ice.

In the Andean Region one may observe two very different types of climate with many variations: The *Interandean Valleys* and the *Andean Slopes*, this last being influenced by the Humboldt Cold Current if they are located on the western slopes, or the Amazonic climate if on the eastern slopes. Yet variations in fauna and vegetation are observed in this many subclimatic regions. The changes in temperature and humidity are sharpest in the higher regions in comparison with the lower ones, and greater on the western slopes more than on the eastern, and even greater in the dry places or "Paramos" or in the dry season, than in the forests and humid places and in the rainy season. In places influenced by the *Oceanic* or *Western Climate*, variation in climatic conditions is larger than in those influenced by the *Eastern* or *Amazonic Climate*. We have good reason to say that Ecuador is a country with extreme climatic changes that determine environmental variations and limit the dispersion of insects and their habitats and biotopic habits. So, as each subregion and each climatic variation has an exclusive population of insects, entomologists are forced

to make a complete study of bioclimatic and meteorologic factors to explain the presence of a population in one location and during one season and its disappearance in the next, and the appearance of another population instead; then the sudden reappearance of the lost population as soon as rains ensue or recede. The cold dry season in the Southern Subregion of the Coastal Plain, for example, forces species to hibernate that are abundant during the rainy season and not a single specimen is seen until the humidity and temperature are again optimum. For example, not a single specimen of *Haemagogus* mosquitoes is found in the dry season in the Coastal Plain, in the Northern Subregion and the Manglaralto-Salango Sub-Subregion, where due to the presence of rain forests, they can be found throughout the year. Yet species differ in this genus and the ones that live in rain forests (*H. soperi*) are not found in the places where rain season-only species appear, such as *H. panarchys*, that disappears in hibernation as soon as the dry season ensues.

The Ecuadorian Center for Entomological Research maintained for 4 consecutive years a high altitude laboratory at Cuenca (2,500 meters above sea level) to study high altitude mosquitoes in their environment, and a field station at Zhurucuchu Lake (3,500 meters) in "Paramo" country. Many important observations have been recorded. These are far from complete but we have concluded that the subgenus *Phalangomyia* of the genus *Culex* should be considered valid for species restricted to high altitudes in the Andean Alley (above 1,000 meters). These mosquitoes have lived under conditions of anoxia and low barometric pressure for thousands of years, becoming different in size and anatomy. Systematically and ecologically they are different populations of *Culex* that can't be confused with the subgenus *Culex*.

The following species have been recorded in Ecuador up to August 1, 1956:

## CULICIDAE

### ANOPHELINI

*Chagasia bathanus* Dyar, 1928. *Anopheles* (*Stethomyia*) *kompfi* Edwards, 1930; *acanthotorynus* Komp, 1937; *A. (Anopheles) pseudopunctipennis rivadeneirai* Levi-Castillo, 1945; *eiseni* Coquillett, 1902; *matogrossensis* Lutz & Neiva, 1911; *perassui* Dyar & Knab, 1908; *A. (Lophopodomyia) squamifemur* Antunes, 1937; *vargasi* Gabaldon, Cova-Garcia & Lopez, 1941; *gomezdeltorrei* Levi-Castillo, 1956; *A. (Arribalzaga) shannoni* Davis, 1931; *punctimacula* Dyar & Knab, 1906; *apicimacula* Dyar & Knab, 1906; *neomaculipalpus* Curry, 1933; *mediopunctatus* Theobald, 1903; *A. (Nyssorhynchus) albimanus* Wiedemann, 1821; *darlingi* Root, 1926; *argyritarsis argyritarsis* Robineau Desvoidy, 1827; *oswaldoi* Peryassu, 1922; *rangeli* Gabaldon, Cova-Garcia & Lopez, 1940; *strodei* Root, 1926; *A. (N.)* sp. n.; *A. (Kerteszia) boliviensis* Theobald, 1905; *bambusicolus* Komp, 1937; *neivai* Howard, Dyar & Knab, 1912; *homunculus* Komp, 1937.

### CULICINI

*Culex (Culex) nigripalpus* Theobald, 1901; *coronator camposi* Dyar, 1925; *coronator coronator* Dyar & Knab, 1906; *corniger* Theobald, 1903; *mollis* Dyar & Knab, 1906; *chidestri* Dyar, 1921; *levicastilloi* Lane, 1945; *pipiens quinquefasciatus* Say, 1823; *maracayensis* Evans, 1923; *C. (Carrollia) bihaicolus* Dyar & Nuñez-Tovar, 1927; *babahoyensis* Levi-Castillo, 1953; *infoliatum* Bonne Wepster & Bonne, 1920; *iridescent* Lutz, 1904; *metempsychus* Dyar, 1921; *secundus* Bonne Wepster & Bonne, 1920; *urichii* Coquillett, 1906; *C. (C.)* sp. n.; *C. (Microculex) chryselatus* Dyar & Knab, 1919; *stonei* Lane & Whitman, 1943; *imitator* Theobald, 1903; *C. (Aedinus) amazonensis* Lutz, 1904; *C. (Lutzia) allostigma* Howard, Dyar & Knab, 1915; *bigoti* Bellardi, 1861; *C. (Tinolestes) conservator* Dyar & Knab, 1906; *originator* Gordon & Evans, 1922; *C. (Melanoconion) manaosensis* Evans, 1924; *madinensis* Senevet, 1936; *conspirator* Dyar & Knab, 1906; *spissipes* Theobald, 1903; *comatus* Senevet & Abonnenc, 1939; *albinensis* Bonne Wepster & Bonne, 1920; *innovator* Evans, 1924; *plectoporce* Root, 1927; *iolambdis* Dyar, 1918; *aikenii* Aiken, 1906; *bastagarius* Dyar & Knab, 1906; *distinguendus* Dyar, 1928; *dunni* Dyar, 1918; *educator* Dyar & Knab, 1906; *eastor* Dyar, 1920; *erraticus* Dyar & Knab, 1905; *elevator* Dyar & Knab, 1906; *hesitator* Dyar & Knab, 1907; *inhibitor* Dyar & Knab, 1906; *pilosus* Dyar & Knab, 1906; *phlogistus* Dyar, 1920; *putumayensis* Matheson, 1934; *taeniopus* Dyar & Knab, 1907; *theobaldi* Lutz, 1905; *C. (Phalangomyia) articularis* Philippi, 1865; *quitensis* Levi-Castillo, 1953; *azuayus* Levi-Castillo, 1954.

*Mansonia (Mansonia) titillans* Walker, 1878; *indubitans* Dyar & Shannon, 1924; *pseudotitillans* Theobald, 1901; *humeralis* Dyar & Knab, 1916; *wilsoni* Barretto & Coutinho, 1944; *M. (Rhynchotaenia) nigricans* Coquillett, 1904. *Aedeomyia squamipennis* Lynch-Arribalzaga, 1878. *Orthopodomyia fascipes* Coquillett, 1905. *Psorophora (Psorophora) ciliata* Fabricius, 1794; *P. (Janthinosoma) ferox* Humboldt, 1820; *lutzi* Theobald, 1901; *cyaneus* Coquillett, 1902; *P. (Grabhamia) confinnis* Lynch-Arribalzaga, 1891; *cingulata* Fabricius, 1805. *Uranotaenia aequatorianna* Levi-Castillo, 1953; *geometrica* Lutz, 1901; *lowii* Theobald, 1901; *leucoptera* Theobald, 1907; *pulcherrima* Lynch-Arribalzaga, 1891; *saphirina* Osten-Sacken, 1868.

### AEDINI

*Aedes (Stegomyia) aegypti* Linneus, 1762 (Now not present); *A. (Ochlerotatus) camposanus* Dyar, 1918; *angustivittatus* Dyar & Knab, 1907; *milleri* Dyar, 1922; *scapularis* Rondani, 1848; *A. (O.)* n. sp.; *A. (Kummyia) serratus* Theobald, 1901; *A. (Culicelsa) taeniorhynchus* Wiedemann, 1821; *A. (Gualteria) terreus metoecopus* Dyar, 1925; *leucotaeniatus* Komp, 1938; *fluviatilis* Lutz, 1904; *leucocelaenus clarki* Galindo, Carpenter & Trapido, 1952; *A. (Howardina) quadrivittatus* Coquillett, 1902; *albonotatus* Coquillett, 1906. *Haemagogus (Haemagogus)*

*spgazzinii janthinomys* Dyar, 1921; *H. (Longipalpifer) panarchys* Dyar, 1921; *soperi* Levi-Castillo, 1955; *garciai* Levi-Castillo, 1956.

#### TOXORHYNCHITINI

*Toxorhynchites hypoptes* Knab, 1907; *aequatoriannus* Levi-Castillo, 1953; *haemorrhoidalis superbus* Dyar & Knab, 1906; *theobaldi* Dyar & Knab, 1906; *bambusicola* Lutz & Neiva, 1913; *T. sp. n.*

#### SABETHINI

*Sabethes (Sabethes) bipartipes* Dyar & Knab, 1906; *cyaneus* Fabricius, 1805; *S. (Sabethoides) chloropterus* Humboldt, 1820; *S. (Sabethinus) fabricii* Lane & Cerqueira, 1942; *identicus* Dyar & Knab, 1907. *Limatus andinus* Levi-Castillo, 1954; *guayasi* Levi-Castillo, 1954. *Trichoprosopon (Trichoprosopon) digitatum* Rondani, 1848; *compressum* Lutz, 1905; *andinus* Levi-Castillo, 1953; *Trichoprosopon (Hyloconops) cotopaxensis* Levi-Castillo, 1953; *lanei* Antunes, 1937; *evansae* Antunes, 1942; *Trichoprosopon (Vonplessenia) vonplesseni* Dyar & Knab, 1906. *Wyeomyia (Wyeomyia) aphobema aequatorialis* Levi-Castillo, 1952; *scotinomus* Dyar & Knab, 1907; *amazonica* Levi-Castillo, 1954; *codiocampa* Dyar & Knab, 1907; *aequatorianna* Levi-Castillo, 1954; *melanopus* Dyar, 1919; *W. (Dendromyia) personata* Lutz, 1904; *chalcocephala* Dyar & Knab, 1906; *complosa* Dyar, 1928; *kerri* Del Ponte & Cerqueira, 1938; *melanocephala* Dyar & Knab, 1906; *W. (D.) sp. n. (1); sp. n. (2); sp. n. (3); sp. n. (4); Phoniomyia splendida* Bonne Wepster & Bonne, 1919; *lassalli* Bonne Wepster & Bonne, 1921; *esmeraldas* Levi-Castillo, 1955.

#### SIMULIIDAE

*Simulium equadoriensis* Enderlein, 1934; *exiguum* Roubaud, 1906; *escomeli* Roubaud, 1906; *quadrivittatum* Loew, 1862; *dinellii* Joan, 1912; *ignescens* Roubaud, 1906; *metallicum* Bellardi, 1859; *amazonicum* Goeldi, 1905; *spinifer* Knab, 1914; *incrustatum* Lutz, 1910; *martinezi* Vargas, 1943; *lutzianum* Pinto, 1931.

#### PHLEBOTOMUS

*Phlebotomus camposi* Rodriguez, 1952; *leopoldoi* Rodriguez, 1953; *guayasi* Rodriguez, 1956; *dysponetus* Fairchild & Hertig, 1952; *gomezi* Nitzulescu, 1931; *barretto* Mangabeira, 1942; *apicalis* Floch & Abonnenc, 1943; *lanei* Barretto & Coutinho, 1941; *trapidoi* Fairchild & Hertig, 1952; *shannoni* Dyar, 1929; *cayenensis* Floch & Abonnenc, 1941; *nordestinus* Mangabeira, 1942; *P. sp. A; sp. B.*

#### CULICOIDES

*Culicoides alahialinus* Barbosa, 1952; *pusillus* Lutz, 1913; *guttatus* Coquillett, 1904; *rozeboomi contubernalis* Ortiz & Leon, 1955; *ocumarensis* Ortiz, 1950; *bricenoi* Ortiz, 1951; *paraensis* Goeldi, 1904; *furens* Poey, 1853; *limonensis* Ortiz & Leon, 1955; *limai* Barretto, 1944; *leoni* Barbosa, 1952; *debilipalpis equatoriensis* Barbosa, 1952; *insinuatus* Ortiz & Leon, 1955; *transferrans* Ortiz, 1953; *camposi* Ortiz & Leon, 1955; *pifanoi blasapambensis* Ortiz & Leon, 1955; *rangeli* Ortiz, 1952; *flochabonnenci* Ortiz & Mirsa, 1952; *phlebotomus* Williston, 1896; *propriipennis* Macfie, 1948; *stigmalis* Coquillett, 1902; *diabolicus* Hoffmann, 1925; *diminutus* Barbosa, 1951; *venezuelensis* Ortiz & Mirsa, 1950.

This is still an incomplete list. There are many new species listed only as n.sp. to be described in due time and much material to be determined. Yet this list gives an idea of what has been done in the last 10 years by the Ecuadorian Center for Entomological research and its staff, working specially on Diptera that have a sanitary interest, because they are proven, or potential vectors of disease. The studies on Diptera of Ecuador are limited in scope and, with the exception of Culicidae, have been restricted to a small territory, because of transport difficulties and the restricted collecting of many species due to impassable roads during the rainy season, especially in the rural areas. However, we have tried hard and are now laboring to bequeath to coming generations the results of our work, and to open the way to further research work in the near future, as we form a new generation of entomologists in our technical schools, and prepare them for the great work ahead to be done as time goes by, and as new roads permit further penetration of virgin territories not yet explored by our teams.





# Inventaire des Arthropodes d'Intérêt Médical et Vétérinaire dans les Territoires Français du Pacifique Sud

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## RÉSUMÉ

Dans cet essai d'inventaire de la faune entomologique pathogène des territoires français du Pacifique: Polynésie (Marquises, Société, Tuamotu, Australes, Wallis) et Mélanésie (Nouvelles-Hébrides, Loyauté, Nouvelle-Calédonie) nous citons 162 espèces appartenant à 91 genres et 49 familles en indiquant brièvement leur répartition géographique et leur rôle en pathologie humaine et animale.

Un nombre restreint d'entre elles transmettent des affections graves: paludisme, endémique aux Nouvelles-Hébrides uniquement (vecteur: *Anopheles farauti*); filariose humaine endémique en Polynésie (vecteur: *Aedes polynesiensis*) avec de rares cas pathogènes en Nouvelle-Calédonie (vecteur: *Aedes vigilax*) et aux Nouvelles-Hébrides; filariose canine à Tahiti et en Nouvelle-Calédonie (vecteur: *Culex annulirostris*); dengue épidémique (vecteurs: *Aedes polynesiensis* à Tahiti; *Aedes scutellaris* aux Nouvelles-Hébrides; *Aedes aegypti* en Nouvelle-Calédonie?) peste en Nouvelle-Calédonie (vecteur: *Xenopsylla cheopis*) paraissant éteinte depuis 1942; typhus murin (vraisemblablement le même vecteur): cas isolés en Nouvelle-Calédonie; piroplasmose bovine localisée à Tahiti (vecteur: *Boophilus annulatus*).

Du point de vue hygiène humaine, la famille des Culicidae, avec les genres *Anopheles*, *Aedes* et *Culex*, est la plus importante; du point de vue zootechnique, c'est celle des Ixodidae (genre: *Boophilus*). Notre connaissance des Arthropodes parasites de l'homme et des animaux domestiques en Océanie française est encore très fragmentaire et nous espérons que le présent inventaire suscitera de nouvelles recherches.

## INTRODUCTION

Si incomplète que soit encore notre connaissance de la faune entomologique d'intérêt médico-vétérinaire dans le Pacifique Sud, quelques caractères s'en dégagent: 1. La pauvreté relative en espèces, surtout en Polynésie (Établissements Français d'Océanie), souvent compensée par une forte densité des individus; 2. L'importance des espèces introduites par rapport à l'élément endémique. 3. Le nombre relativement restreint d'affections graves transmises par les Arthropodes et leur localisation: paludisme aux Nouvelles-Hébrides seulement, peste en Nouvelle-Calédonie (semblant actuellement éteinte), filariose, pathogène surtout en Polynésie, dengue épidémique, typhus murin (quelques cas en Nouvelle-Calédonie), piroplasmose apparemment limitée à Tahiti.

Mais dans le domaine médical et vétérinaire, malgré l'impulsion donnée aux recherches par la deuxième guerre mondiale, bien des études sur l'épidémiologie des maladies à vecteur animal restent à entreprendre et nous ne possédons trop souvent que des données imprécises et fragmentaires.

Du point de vue zoogéographique, la faune entomologique médicale des territoires français du Pacifique semble peu homogène, chaque archipel présentant des caractères particuliers. Les deux régions occidentale ou mélanésienne et orientale ou polynésienne se différencient nettement. Dans la partie occidentale de ces territoires: Nouvelle-Calédonie, Loyauté, Nouvelles-Hébrides, la faune entomologique présente des affinités avec celle d'Australie et de Nouvelle-Guinée; il y a aussi un apport indo-malais. Dans la partie orientale: îles Wallis et Futuna, îles de la Société, Australes, Tuamotu, Gambier et Marquises, elle s'appauvrit considérablement et s'apparente à la faune orientale (indo-malaise). Dans les deux régions ont été introduites en nombre important des espèces cosmopolites ou cosmotropicales.

## APTÉRYGOTES

Aucun Aptérygote n'a été signalé comme présentant un intérêt médical et vétérinaire dans les territoires français du Pacifique.

## ORTHOPTÈRES

Seuls les *Blattodea* ou *Blattaria* nous intéressent. Les blattes domestiques peuvent héberger de nombreux organismes pathogènes: bactéries, kystes d'amibes, oeufs d'helminthes; certaines sont les hôtes intermédiaires de divers Nématodes et Acanthocéphales. Dans des cas de pullulation intense, les *Periplaneta* et *Blattella* peuvent s'attaquer à l'homme (enfants notamment), mordillant la peau et les poils, rongant les croûtes et élargissant les plaies. De tels cas, heureusement peu fréquents, nous ont été rapportés de Nouvelle-Calédonie et des Nouvelles-Hébrides (Santo).

*Blattella germanica* (L.): espèce cosmopolite, surtout abondante aux Nouvelles-Hébrides.

Hôte intermédiaire de *Gongylonema*, nématodes parasites d'animaux domestiques, du rat et, parfois, de l'homme.

*Periplaneta americana* (L.): cosmopolite, répandue dans tout le Pacifique Sud; hôte intermédiaire de *Gongylonema* et de l'Acanthocéphale *Moniliformis moniliformis* qui vit à l'état adulte dans l'intestin des rats, en particulier en Nouvelle-Calédonie.

*P. australasiae* (F.): associée à l'espèce précédente et ayant le même rôle pathogène.

*P. brunnea* (Burmeister): Nouvelle-Calédonie, Loyauté, Nouvelles-Hébrides, Marquises.

*Pycnoscelus surinamensis* (L.): héberge la larve du nématode *Oxyspirura mansoni*, le ver des yeux des volailles, connu en Nouvelle-Calédonie et aux E.F.O. Cosmotropicale.

Quatre espèces d'Hyménoptères s'attaquent aux *Periplaneta* (cf. p. 5).

## HÉMIPTÈRES

## 1) CIMICIDAE

*Cimex lectularius* L.: la punaise des lits. Cosmopolite et répandue dans tous les territoires français du Pacifique.

*C. rotundatus* Signoret: cosmopolite, signalée des Nouvelles-Hébrides.

*Oeciacus* sp. (?): punaise des nids d'hirondelles en Nouvelle-Calédonie.

## 2) REDUVIIDAE

Les Triatominae, hématophages et vecteurs de la trypanosomiase sud-américaine (maladie de Chagas) ne sont pas connus dans nos territoires. Il existe par contre plusieurs espèces de Reduviidae prédatrices d'insectes et pouvant infliger une piqûre douloureuse lorsqu'on les saisit imprudemment, de même que des Hydrocorises (Notonectidae, Belostomatidae). Nous avons observé plusieurs espèces d'Emesitae en Nouvelle-Calédonie, dont une capturant de petits insectes dans les maisons.

## 3) BELOSTOMATIDAE

*Belostoma insulanum* Montandon, de Nouvelle-Calédonie, est une punaise aquatique géante s'attaquant parfois aux poissons et même aux jeunes canards que ses piqûres peuvent faire périr.

## ANOPOLOURES

## 1) PEDICULIDAE

*Pediculus humanus* L. Pou de tête et pou de corps humains; cosmopolite.

*Pthirus pubis* L.: pou du pubis; également cosmopolite.

## 2) HAEMATOPINIDAE

*Polyplax spinulosa* Brumeister. Pou de rat; vecteur du typhus murin de rat à rat. Sur *Rattus rattus alexandrinus* en Nouvelle-Calédonie. Cosmopolite.

*Hoplopleura oenomydis* Ferris. parasite des rats, en particulier le Rat maori (*Rattus exulans*) aux Marquises.

*Haematopinus suis* L. (et var. *adventicius* Neumann): pou de porc. Cosmopolite: Nouvelle-Calédonie, Loyauté, Nouvelles-Hébrides, vraisemblablement Etablissements Français de l'Océanie.

*Linognathus setosus* (Olfers): pou de chien. Nouvelle-Calédonie.

## MALLOPHAGES

Les espèces qui ont été signalées sur les animaux domestiques en Océanie sont cosmopolites; leur importance du point de vue zootechnique paraît assez faible.

## AMBLYCERA

## 1) GYROPIDAE (Introduits en Océanie avec les cobayes)

*Gyropus ovalis* Nitzsch et *Gliricola porcelli* (L.) recueillis par nous sur cobayes à Nouméa (Nouvelle-Calédonie).

## 2) BOOPIDAE (Ectoparasites de marsupiaux et de carnivores)

*Heterodoxus spiniger* (Enderlein) observé par nous sur chien à Lifou (Loyauté) peut pulluler sur les animaux mal soignés.

## 3) MENOPONIDAE (Ectoparasites d'oiseaux)

*Eomenacanthus stramineus* (Nitzsch): sur poulet et dindon en Nouvelle-Calédonie.

*Menopon gallinae* (L.): sur poulet, canard, pintade, pigeon et dindon en Nouvelle-Calédonie et à Tahiti (E.F.O.).

*Colpocephalum turbinatum* Denny: sur pigeon en Nouvelle-Calédonie.

## ISCHNOCERA

## 1) TRICHODECTIDAE (Ectoparasites de Mammifères)

*Trichodectes canis* (de Geer): sur chien, Nouvelle-Calédonie, Etablissements Français d'Océanie.

*Damalinea (Bovicola) caprae* (Gurlt, 1843): sur chèvre. Nouvelle-Calédonie et dépendances.

*D. bovis* (L.), *D. ovis* (L.), *D. equi* (L.): respectivement sur boeuf, mouton, cheval.

*Felicola subrostrata* (Nitzsch): sur chat. Vraisemblablement présents dans nos territoires.

## 2) PHILOPTERIDAE (Ectoparasites d'oiseaux)

*Columbicola columbae* (L.): sur pigeon. Nouvelle-Calédonie.

*Lipeurus caponis* (L.): sur poulet et paon. Nouvelle-Calédonie et Etablissements Français de l'Océanie.

*Cuclotogaster heterographus* (Nitzsch): sur poulet. Nouvelle-Calédonie.

*Oxylipaeus polytrapezius* (Burmeister): sur dindon (Nouvelle-Calédonie).

*Goniocotes gallinae* (de Geer): sur poulet et dindon. Nouvelle-Calédonie.

*G. bidentatus* Scopoli: sur pigeon et tourterelle. Nouvelle-Calédonie.

*Goniodes dissimilis* Nitzsch: sur poulet. Nouvelle-Calédonie.

*Goniodes pavonis* (L.): sur paon. Nouvelle-Calédonie.

*Chelopistes meleagridis* (L.): sur dindon. Nouvelle-Calédonie.

## LÉPIDOPTÈRES

A notre connaissance, aucun Lépidoptère n'a été signalé comme présentant un intérêt médical dans les territoires français du Pacifique. Il existe cependant, au moins en Nouvelle-Calédonie, quelques espèces dont les chenilles portent des poils urticants.

## COLÉOPTÈRES

Nous ne possédons pas de renseignements sur le rôle pathogène éventuel de cet ordre en Océanie française.

## 1) STAPHILINIDAE

Deux espèces de *Paederus* (*P. cruenticollis* var. *cingulatus* McLeay et *P. lacordairei* Perroud) sont connues de Nouvelle-Calédonie. Des dermatites vésiculaires causées par l'écrasement de ces insectes sur la peau ont été signalées, notamment en Indochine et en Australie. Nous avons noté également des punaises vésicantes de la famille des Cydnidae.

## 2) CARABIDAE

Quelques grosses espèces de Nouvelle-Calédonie peuvent projeter un liquide corrosif émis par des glandes anales. Il en est de même pour certaines blattes du genre *Cutilia*.

## 3) DYTISCIDAE

Prédateurs aquatiques détruisant parfois les larves de moustiques (par ex. *Rhantus punctatus* Fourcroy en Nouvelle-Calédonie). Par contre de grosses espèces comme *Cybister*

*tripunctatus* Olivier en Nouvelle-Calédonie s'attaquent aux alevins et peuvent nuire à la pisciculture.

## HYMÉNOPTÈRES

Chez la plupart des Aculéates, la femelle pourvue d'un aiguillon venimeux peut infliger une piqûre plus ou moins douloureuse. Seules les formes sociales sont capables de provoquer chez l'homme des accidents sérieux par la multiplicité de leurs attaques.

### 1) SPHECIDAE

La piqûre des *Sceliphron*, connus de tous les territoires français du Pacifique, est réputée très douloureuse. Ce sont des chasseurs d'araignées.

### 2) VESPIDAE

*Polistes olivaceus* (de Geer): Nouvelle-Calédonie, Nouvelles-Hébrides, Etablissements Français de l'Océanie. Guêpe sociale très agressive lorsqu'on approche de ses nids en papier à cellules hexagonales, fixés par un pédoncule aux branches des arbres, aux buissons ou à l'intérieur des hangars.

### 3) FORMICIDAE

*Solenopsis geminata* var. *rufa* Jerdon ou "fire ant", répandue en Nouvelle-Calédonie, pique sévèrement lorsqu'on dérange sa fourmilière.

### 4) APIDAE

*Apis mellifica* L. et var. *ligustica* Spinola existe à l'état sauvage dans les îles françaises du Pacifique; elle peut provoquer des accidents par la multiplicité de ses piqûres lorsqu'on s'approche trop près de ses ruches.

### 5) HYMÉNOPTÈRES ENTOMOPHAGES

On a fondé sur eux de grands espoirs pour la lutte biologique contre les insectes d'intérêt économique dans le Pacifique, surtout aux Hawaii.

*Ampulex compressa* (F.) est prédateur de blattes (*Periplaneta*) en Nouvelle-Calédonie.

*Evania appendigaster* L. et *E. impressa* Schlett. sont parasites des oothèques des *Periplaneta*; de même un *Eulophidae* cosmopolite: *Tetrastichus hagenowii* (Ratzburg), signalé par nous de Nouvelle-Calédonie.

*Australomalotylus rageaui* Risbec, Encyrtidae, parasite les pupes de *Sarcophaga* en Nouvelle-Calédonie.

Les Pompilidae, représentés dans tous nos territoires, sont prédateurs d'araignées.

## DIPTÈRES

### I. NÉMATOCÈRES

#### 1) PSYCHODIDAE

Les phlébotomes ne sont pas connus dans nos territoires; les Psychodinae sont fréquents mais on n'a signalé aucune espèce pathogène.

#### 2) CERATOPOGONIDAE

Ils n'ont été étudiés que dans les îles de la Société et Marquises: genres *Atrichopogon*, *Culicoides*, *Dasyhelea*, *Forcipomyia*, *Lasiohelea*, *Stilobezzia* et *Styloconops*. La plupart des espèces paraissent endémiques. On ne leur connaît pas de rôle vecteur bien que certains paraissent susceptibles de piquer l'homme. Leur biologie est pratiquement inconnue mais, en Nouvelle-Calédonie, nous n'avons pas observé d'espèces anthropophiles.

#### 3) SIMULIIDAE

Cette famille paraît pauvre en espèces dans le Pacifique français et on ne lui connaît pas de rôle pathogène en dehors de ses piqûres, notamment aux Marquises, où *Simulium buissoni* Roubaud ("nono") se gorge avec avidité sur l'homme.

*S. buissoni*, var. *gallinum* Edwards; Marquises; se nourrit sur volailles.

*S. adamsoni* Edwards et *S. mumfordi* Edw.: Marquises.

*S. tahitiense* Edw., *S. cheesmanae* Edw. et *S. oviceps* Edw.: Tahiti, ne semblent pas agressives.

*S. jolyi* Roubaud: Nouvelles-Hébrides.



*S. ornatipes* Skuse: Nouvelle-Calédonie et île des Pins; se pose volontiers sur la peau mais nous ne l'avons pas observée en train de se gorger. Cette espèce, très répandue en Nouvelle-Calédonie, n'était connue jusqu'à présent que d'Australie.

#### 4) CULICIDAE

En Océanie française, les Culicidae sont vecteurs d'affections graves et très répandues, endémiques ou épidémiques: paludisme localisé aux Nouvelles-Hébrides, dengue et filariose dans la plupart des îles. Des espèces côtières particulièrement agressives, peuvent présenter une densité telle qu'elles rendent ces régions inhabitables: c'est le cas de certains *Aedes* et, à un moindre degré, des *Culex* et *Anophèles*.

*Anopheles* (*Myzomyia*) *farauti* Laveran: limité aux Nouvelles-hébrides, sauf l'île de Futuna. Vecteur du paludisme dans ces îles, vraisemblablement aussi de la filariose humaine.

*Aedes* (*Mucoides*) *alternans* Westwood: Nouvelle-Calédonie, Loyauté (Ouvéa). Anthropophile, mais rarement agressive en Nouvelle-Calédonie. Larves prédatrices d'autres larves de moustiques, vivant en zone côtière.

A. (M.) *kermorganti* Laveran: Nouvelle-Calédonie. Variété ou synonyme du précédent?

A. (*Ochlerotatus*) *vigilax* Skuse. Nouvelle-Calédonie et dépendances, Nouvelles-Hébrides. Espèce côtière, très agressive, vectrice de filariose humaine (*Wuchereria bancrofti*, var. apériodique).

A. (O.) *edgari* Stone et Rosen: Tahiti; vecteur de *W. bancrofti*, var. *pacifica* Manson-Bahr.

A. (*Aëdimorphus*) *vexans* var. *nocturnus* Theo. Nouvelle-Calédonie, Loyauté, Nouvelles-Hébrides. Anthropophile et souvent exophile; larves dans les prairies inondées.

A. (*Stegomyia*) *aegypti* L. Cosmotropical. Très anthropophile; incriminé dans la transmission de la dengue en Nouvelle-Calédonie.

A. (S.) *polynesiensis* Marks. Polynésie (E.F.O. and Wallis); anthropophile et principal vecteur de la filariose humaine à Tahiti, ainsi que de la filariose canine (*Dirofilaria immitis*) et de la dengue.

A. (S.) *scutellaris* Walker: Nouvelles-Hébrides. Anthropophile, vecteur de la filariose de Bancroft et de la dengue.

A. (S.) *pernotatus* Farner et Bohart: Nouvelles-Hébrides.

A. (S.) *tongae* Edwards: Nouvelles-Hébrides et Tahiti (?).

A. (*Finlaya*) *notoscriptus* Skuse. N. Calédonie et dépendances; anthropophile, surtout exophile.

A. (F.) *samoanus* (Grünberg): Wallis; pique la nuit.

A. (*Aedes*) *lineatus* Taylor: N. Hébrides.

A. (*Geoskusea*) *daggyi* Stone et Bohart: Nouvelles-Hébrides.

*Culex* (*Culex*) *annulirostris* Skuse: Nouvelle-Calédonie et dépendances, Nouvelles-Hébrides, E.F.O. Amphophile, vecteur de la filariose canine à Tahiti et, vraisemblablement, en Nouvelle-Calédonie.

C. (C.) *pipiens fatigans* (Wiedemann); cosmotropical, d'introduction récente en Océanie comme *Aedes aegypti*. Amphophile, à activité surtout nocturne. En Nouvelle-Calédonie il est souvent ornithophile. Il paraît un mauvais vecteur de filariose dans nos territoires.

C. (C.) *pipiens australicus* Dobrotworsky et Drummond: récoltée par nous en Nouvelle-Calédonie et identifiée par Miss Marks.

C. (C.) *pacificus* Edwards: décrit des Nouvelles-Hébrides.

C. (C.) *iyengari* Mattingly et Rageau, 1958: voisin de *C. pacificus* et de *C. pervigilans* de Nouvelle-Zélande: Nouvelle-Calédonie, îles Loyauté (Lifou), île des Pins.

C. (C.) *sitiens* Wiedemann: Nouvelle-Calédonie, Nouvelles-Hébrides, Wallis, Tahiti. Espèce côtière, anthropophile mais ne paraissant pas un bon vecteur de filariose; vaste répartition.

C. (C.) *basicinctus* Edw.: Nouvelle-Calédonie, Nouvelles-Hébrides.

C. (C.) *atriceps* Edw. et C. (C.) *litoralis* Bohart: Tahiti.

C. (C.) *marquesensis* Stone & Rosen: Marquises.

- C. (C.) bitaeniorhynchus* Giles: récemment signalé de Nouvelle-Calédonie par Laird (1954).  
*C. (Neoculex) cheesmanae* Mattingly et Marks: vient d'être décrit de Nouvelle-Calédonie.  
*C. (Mochthogenes) femineus* Edw.: Nouvelles-Hébrides.  
*C. (Lophoceratomyia) hilli buxtoni* Edw. et *C. (L.) solomonis* Edw.: Nouvelles-Hébrides.  
*C. (L.) fraudatrix* Theo.: archipel des Bélep (île Art), dépendance de la Nouvelle-Calédonie.  
*Taeniorhynchus (Coquillettidia) crassipes* v.d.Wulp: Nouvelles-Hébrides, Nouvelle-Calédonie (?).  
*T. (C.) xanthogaster* Edw.: Nouvelles-Hébrides, Nouvelle-Calédonie; anthropophile mais non connu comme vecteur de filariose. Les genres *Taeniorhynchus*; *Tripteroides* et *Uranotaenia* n'ont pas de représentants en Polynésie.  
*Tripteroides (Mimeteomyia) caledonica* Edw.: Nouvelle-Calédonie; Nouvelles-Hébrides(?). Non anthropophile.  
*T. (N.) melanesiensis* Belkin: Nouvelle-Calédonie et dépendances, Nouvelles-Hébrides.  
*T. (M.) folicola* Belkin: Nouvelles-Hébrides.  
*T. (M.) coheni* Belkin: Nouvelles-Hébrides (?); *T. solomonis* Edw.; Nouvelle-Calédonie (?).  
*Uranotaenia tibialis* Taylor: Nouvelles-Hébrides.  
*Toxorhynchites brevipalpis* Theo.: récemment introduit à Tahiti par D. D. Bonnet pour la lutte biologique contre *Aedes polynesiensis*; ses larves dévorent les larves des autres Culicidae mais les femelles ne sont pas hématophages.

## II. BRACHYCÈRES

### 1) TABANIDAE

Une révision de ces Diptères dans le Pacifique Sud est en cours (par I. M. Mackerras et J. Rageau); aucune espèce n'est signalée des Etablissements Français d'Océanie. Rôle pathogène inconnu.

*Tabanus expulsus* Walker: Nouvelles-Hébrides (Aneityum-Tanna).

*Dasybasis rubricallosa* (Ricardo): Nouvelle-Calédonie et dépendances; espèce côtière très agressive.

*Cydistomyia caledonica* (Ricardo): Nouvelle-Calédonie.

*C. lifuensis* (Bigot): Loyauté (Lifou).

*Cydistomyia n. sp.*: 8 espèces nouvelles sont en cours de description, toutes en provenance de la Nouvelle-Calédonie sauf une de l'île des Pins: Les *Cydistomyia* ne paraissent pas anthropophiles.

*Philoliche neocaledonica* Mégnin: Nouvelle-Calédonie et île des Pins; a été accusée par Mégnin (1878) de propager une épidémie de charbon à l'île des Pins, mais ne semble pas se gorger sur l'homme. 2 espèces nouvelles de *Philoliche* de Nouvelle-Calédonie sont en cours de description.

### 2) HIPPOBOSCIDAE

*Hippobosca equina* L.: Nouvelle-Calédonie et dépendances, Nouvelles-Hébrides, E.F.O. Introduite à la fin du siècle dernier et actuellement très commune sur les bovidés et les équidés.

### 3) MUSCIDAE

*Musca domestica* L. et var. *vicina* Macquart: la mouche domestique; commune dans tout le Pacifique, sa pullulation la rend gênante dans certaines îles.

*Musca sorbens* Wied.: Nouvelle-Calédonie, Nouvelles-Hébrides.

*Muscina stabulans* (Fallen): Nouvelles-Hébrides.

*Stomoxys calcitrans*: le seul Muscidae piqueur en Océanie française. Harcèle de ses piqûres les chevaux, boeufs, porcs, chiens et même l'homme; accusée d'avoir transmis le charbon au cours d'une épidémie à l'île des Pins en 1878.

### 4) CALLIPHORIDAE ET SARCOPHAGIDAE

Dans les îles françaises du Pacifique, aucun cas de myiase n'a été publié, bien que l'on y retrouve des espèces australiennes de Calliphoridae agents de graves myiases, notamment chez les moutons, en Australie.

- Calliphora* (*Proekton*) *aruspex* Bezzi: Nouvelles-Hébrides.  
*C.* (*Neopollenia*) *dichromata* Bigot: Nouvelle-Calédonie. *C. australis* et *C. stygia* se retrouvent vraisemblablement en Nouvelle-Calédonie.  
*C.* (*Anastellorhina*) *augur* (F.): id.  
*C. vicina* R.D.: id.  
*Microcalliphora varipes* (Macquart): existe en Nouvelle-Calédonie et dépendances.  
*Chrysomya megacephala* F.: Nouvelles-Hébrides (Santo).  
*Ch. rufifacies* (Macquart): Nouvelle-Calédonie, Nouvelles-Hébrides, E.F.O.  
*Lucilia* (*Phoenicia*) *cuprina* Wied.: Nouvelle-Calédonie et dépendances, Nouvelles-Hébrides.  
*L. sericata* Meigen: Nouvelles-Hébrides (Santo).  
*L. calviceps* Bezzi: id.  
*L. metilia* Walker: Nouvelle-Calédonie.  
*Sarcophaga chalcura* Bezzi: Nouvelles-Hébrides.  
*S. haemorrhoidalis* Fallen: Nouvelle-Calédonie.  
*Sarcophaga* sp.: plusieurs espèces non encore identifiées en Nouvelle-Calédonie et dépendances.  
*S. orchidea* Böttcher: Nouvelles-Hébrides.  
*S. taitensis* Schiner: Tahiti.

#### 5) OESTRIDAE AND GASTEROPHILIDAE

Ne paraissent pas avoir été signalés dans les îles françaises du Pacifique, bien qu'ils soient connus d'Australie et des îles Hawaïi.

### APHANIPTÈRES

- Pulex irritans* L.: la puce humaine, introduite aux XVIII-ème et XIX-ème siècles dans tout le Pacifique et signalée des Nouvelles-Hébrides et des E.F.O.; ne paraît pas répandue en Nouvelle-Calédonie.  
*Ctenocephalides felis felis* (Bouché): puce du chien et du chat; cosmopolite, extrêmement commune dans les îles françaises du Pacifique. Ne semble pas un bon vecteur de la peste.  
*C. canis* (Curtis): il est possible que cette espèce ait été introduite dans nos territoires avec des chiens.  
*Xenopsylla cheopis* Rothschild: puce du rat, cosmotropicale. Nouvelle-Calédonie sur *Rattus rattus alexandrinus* et *R. norvegicus*; îles Marquises sur rats. C'est le vecteur classique de la peste dont plusieurs épidémies se sont succédées en Nouvelle-Calédonie entre 1900-1942; transmet également le typhus murin, signalé en Nouvelle-Calédonie par Sanner (1950).

### ARACHNIDES

#### I. ARANEIDES

- Latrodectus hasseltii* (Thorell) (Theridiidae): Nouvelle-Calédonie et dépendances. Seule araignée venimeuse connue de l'Océanie française.

#### II. SCORPIONS

- Hormurus australasiae* (F.): répandu dans tout le Pacifique Sud.  
*H. neocaledonicus* (Simon) *H. sarasini* Kraepelin et *H. caudicula* (Koch): Nouvelle-Calédonie.  
*Isometrus maculatus* de Geer: cosmotropical. Nouvelle-Calédonie et E.F.O.

#### III. ACARIENS

L'acarologie médicale et vétérinaire débute à peine dans les territoires français du Pacifique Sud. Ce sont les familles des Ixodidae et des Sarcoptidae qui présentent le plus d'intérêt.

#### 1) DERMANYSSIDAE

- Dermanyssus gallinae* Redi: ectoparasite des poulets. Nouvelle-Calédonie.  
*Bdellonyssus bursa* Berlese: id.

## 2) LAELAPTIDAE

*Echinolaelaps echidninus* (Berlese) et *Laelaps hawaiiensis* Ewing: sur rats aux Marquises. *Laelaps* sp.: sur rats et souris (*Mus musculus canacorum* Revilliod) en Nouvelle-Calédonie.

## 3) ARGASIDAE (Non signalés dans nos territoires)

## 4) IXODIDAE

*Amblyomma cyprium* Neumann et *A. quasicyprum* Robinson: Nouvelles-Hébrides; biologie inconnue.

*Boophilus annulatus* (Say): E.F.O. (Tahiti); vraisemblablement d'origine américaine; vecteur de piroplasmose bovine ou fièvre du Texas (*Piroplasma bigeminum*).

*Boophilus microplus* (Canestrini): introduit d'Australie en Nouvelle-Calédonie au cours de la deuxième guerre mondiale, c'est un fléau pour le bétail qu'il épuise rapidement, obligeant les éleveurs à utiliser des bains détiquteurs. Cette espèce transmet en Australie des piroplasmoses mais ces maladies n'ont pas été introduites en Nouvelle-Calédonie.

Ses larves peuvent se fixer sur l'homme provoquant des dermatites. Les îles Loyauté, l'île des Pins et les autres territoires français du Pacifique sont encore indemnes de ce dangereux parasite.

*Haemaphysalis bispinosa* Neumann: sur boeuf, cerf, chien, en Nouvelle-Calédonie. Origine australienne ou néo-zélandaise.

*Rhipicephalus sanguineus* Latreille: sur chien, plus rarement chat ou homme. Cosmopolite, fréquente en Nouvelle-Calédonie. Pas de rôle pathogène connu.

## 5) DEMODECIDAE

Le genre *Demodex*, cosmopolite, se retrouve dans les îles françaises du Pacifique.

## 6) TROMBICULIDAE

*Hannemania rouxi* Oudemans: larves vivant enfoncées dans la peau de la rainette dorée d'Australie (*Hyla aurea* Less). Nouvelle-Calédonie.

Aucune espèce d'intérêt médical n'a été signalée des régions qui nous concernent. Un cas de "scrub typhus" a été publié des Nouvelles-Hébrides par Bourdin (1951) mais le vecteur reste à identifier.

## 7) HYDRACHNELLAE

Des larves non identifiées génériquement ont été récoltées sur deux espèces de moustiques en Nouvelle-Calédonie: *Aedes vigilax* et *Culex sitiens*.

## 8) TYROGLYPHIDAE

*Tyroglyphus castellanii* Hirst provoque chez l'homme la dermatite du coprah ("copra itch") dans les îles du Pacifique.

## 9) SARCOPTIDAE

Agents de gales chez l'homme et les animaux domestiques, la plupart sont cosmopolites. Citons:

*Sarcoptes scabiei*, var. *hominis* et *canis* L.: agents de la gale humaine et canine.

*Notoedres cati* (Hering): agent de la gale de la tête du chat. Nouvelle-Calédonie.

*Psoroptes equi* (Hering): agent de la gale du cheval. Des variétés de cette espèce provoquent la gale du mouton, du boeuf, de la chèvre. Nouvelle-Calédonie.

*Cnemidoptes mutans* Robin and Lanquetin: gale des pattes du poulet. Nouvelle-Calédonie et dépendances, Tahiti.

## 10) ANALGESIDAE (et DERMOPHYLIDAE)

Acariens plumicoles, vivant sur les poulets en Nouvelle-Calédonie.

## MYRIAPODES

## 1) DIPLOPODES

Non signalés comme présentant un intérêt médical dans nos territoires.



## 2) CHILOPODES

*Scolopendra morsitans* L.: espèce venimeuse de grande taille dont la piqûre est douloureuse mais ne paraît pas mortelle. Nouvelle-Calédonie (Ribaut, 1923); Marquises (Silvestri, 1939).

*Scolopendra subspinipes* Leach.: rôle pathogène analogue. Répandue dans tout le Pacifique Sud.

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# Les Sclerodermines par Rapport à l'Homme

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## RÉSUMÉ

Il m'a paru intéressant d'attirer l'attention sur une invasion singulière de Scleroderminae que j'ai eu l'occasion d'observer dans deux maisons de Gênes (Italia). Le 22 mars 1956 Mme et M. B. se sont plaints de douloureuses piqûres. Les accidents d'envenimation sont très vivement ressentis. Les troubles locaux, de M. B. sont accompagnés de phénomènes généraux à type anaphylactique, avec malaise, nausées, cephalgie. Mme B. est piquée dans son lit en plusieurs points du corps. Par ailleurs, l'état général n'est pas troublé. L'insecte responsable est *Scleroderma domesticum* Latr. Il vient d'une armoire de bois "vermoulu" placée dans la chambre à coucher de Mme et M. B. Plusieurs femelles y sont capturées à leur sortie des galeries creusées dans le bois par l'*Anobium*. Le 30 mars 1956 à la suite de piqûres nombreuses en différents points du corps infligées à trois membres de la famille V., mon attention a été attirée par l'agent vulnérant qui est une nouvelle espèce de *Cephalonomia*. Plusieurs femelles aptères ont été recoltées dans le rembourrage végétal d'un fauteuil contenant des *Lasioderma*. A la suite de ces cas de piqûres par Scleroderminae nous avons fait une courte révision des observations analogues publiées jusqu'aujourd'hui par les différents auteurs.

Le 22 mars 1956 vint dans mon laboratoire, accompagnée par son mari, Madame B. de Gênes, qui me dit être depuis un certain temps affligée, surtout aux heures nocturnes, par des piqûres fort douloureuses accompagnées par une très ennuyante et insistante démangeaison qui n'était pas limitée exclusivement à la partie piquée, mais étendue à tout le corps, comme si elle eût été affligée par de l'urticaire. Elle présentait en effet sur le dos, à la hauteur de l'épaule droite, de grosses papules indurées, proches les unes des autres et fortement rougeâtres; leur diamètre ne dépassait pas un centimètre; toute la région autour était inflammée et prurigineuse.

La malade me dit même avoir eu auparavant d'autres piqûres sur les bras, sur les cuisses, sur le ventre, c'est-à-dire sur les parties du corps les plus molles et charnues. Excepté l'ennuyeuse démangeaison et une nervosité générale qui se prolongeait depuis plusieurs jours, elle n'accusait pas d'autres troubles particuliers.

Son mari, bien qu'il dormît à côté d'elle, n'avait reçu pendant plusieurs jours aucune piqûre; il avait eu seulement la nuit avant la douloureuse sensation d'une épingle chauffée à blanc qui pénètre dans les chairs. Il me fit voir en effet une papule rougeâtre, endurcie et fort prurigineuse d'un centimètre et demi environs de diamètre. Cette unique piqûre lui avait amené des troubles généraux assez sérieux; une remarquable anorexie, un sens de nausée et un étrange trouble phisio-psychique, troubles que sa femme n'avait jamais accusés, bien qu'elle eut été plusieurs fois fortement piquée.

D'après l'observation de l'insecte en question il me fut très facile de constater qu'il s'agissait d'un *Bethylidae* au plus proprement d'une femelle du *Scleroderma domesticum* Latreille. Dans le but d'étudier de près le cas, je suis allée dans l'appartement infesté, ou, dans la chambre de Mme et M. B. je trouvais une grande armoire diffusement et profondément criblée par les larves de l'*Anobium*. Près des trous de sortie je pus facilement trouver quelques femelles du *Scleroderma domesticum*. Il est étrange que ce microhyménoptère avait tout à fait épargné la fille de deux ans et demi qui avait son lit contre l'armoire en question et le bébé de cinq mois qui avait son berceau tout près de là.

Peu de jours après les faits que je viens d'exposer, la belle-soeur de Mme B. me soumit quelques microhyménoptères qu'elle avait trouvés chez elle et qu'elle soupçonnait être la cause de certaines piqûres fort ennuyeuses qui depuis plusieurs jours tourmentaient Mme et les siens. Cette fois aussi il s'agissait de Sclérodermines et plus précisément de femelles aptères d'une espèce du genre *Cephalonomia*<sup>1</sup>. Très intéressée au cas, je suis allée dans l'appartement infesté et j'ai trouvé dans la pièce qui était le centre d'infection, un fauteuil dont la couverture en peau laissait voir, surtout du côté du dossier et des bras un grand nombre de petits trous réguliers, près desquels je ne tardai pas à trouver les femelles de la *Cephalonomia* en question.

<sup>1</sup>Pour la description voir *Mem. Soc. Ent. It.* 35: 129-132, 1956.

Après avoir démonté le fauteuil, son squelette en bois ne présentait aucun trou qui aurait pu révéler la présence de l'*Anobium*. Dans le rembourrage fait en crin végétal, je pus retrouver des morceaux d'un coléoptère du genre *Lasioderma* (Fam. *Anobidae*) qui, on sait bien, comprend des espèces cosmopolites qui attaquent toute sorte de substance sèches d'origine végétale ou animale. Avec toute probabilité c'était les larves de ce coléoptère, ou peut-être d'un autre, comme le *Stegobium paniceum* L. que la *Cephalonomia* parasitait.

Des trois membres de la famille frappée, un garçon de six ans et demi, la mère et sa grand'mère, les deux premiers présentaient sur le cou des papules endurcies, d'un diamètre inférieur à un centimètre limitées à une région plutôt étroite; la dernière des taches rougeâtres sur les cuisses datait déjà d'une dizaine de jours. Pour ce qui concerne la version sur les troubles qui suivirent la piqûre, tous étaient d'accord à se plaindre d'une très ennuyeuse et persistante démangeaison accompagnée par un état, plus ou moins accentué, de nervosisme général.

Comparées avec les papules du *Scleroderma* elles se présentaient moins enflées et étendues et l'auréole rouge autour de la piqûre plus petite. Les manifestations cutanées ressemblaient donc à celles causées par la piqûre des moustiques ou des puces, tandis que dans le cas du *Scleroderma* celles-ci rappelaient plutôt la piqûre des punaises. Aucun des sujets atteints n'avait accusé de troubles de caractère général, si l'on fait exception, ainsi que j'ai déjà dit, d'un certain état de nervosité causé surtout par l'ennuyeuse et persistante démangeaison. Pour conclure, les effets de la piqûre de la *Cephalonomia* paraissent moins sérieux que ceux du *Scleroderma*.

Bien que dans "Hymenoptera of America north of Mexico—Synoptic Catalog" (1951 page 727) on dit que la *Cephalonomia gallicola* Ashmead pique souvent l'homme, cependant je pense que c'est la première fois, du moins en Italie, que la *Cephalonomia* est considérée sous cet aspect. Nous savons d'autre part que les connaissances à l'égard des *Sclerodermines* par rapport à l'homme, pour ce qui concerne le vieux continent, sont bien pauvres et, l'on peut dire, presque toutes condensées en ces dernières années.

Encore en 1950 on disait: "Plusieurs espèces de *Sclerodermes* sont communes en France, en Europe et en Afrique du Nord, notamment *S. domestica* Latr. A notre connaissance, il n'a jamais été signalé que l'homme ait été parfois attaqué par ces insectes (Mandoul, Bernard, Jacquemin, page 159), bien que l'ancienne littérature eut déjà rapporté des mentions à ce propos. Quant à moi, je pense que l'une des plus anciennes mentions soit celle de Saunders (d'après Westwood 1839 page 170). Cet auteur dit avoir fréquemment trouvé dans les maisons de Prevesa et de Santa Maura (Albania) une espèce de *Scleroderma* (*S. cylindrica*) qui piquait les parties exposées du corps ("by acutely stinging the exposed parts of the body). Ensuite (1880 page 109) il reprend la question en disant avoir lui-même essayé cette désagréable piqûre et en citant en même temps des remarques de Haliday A. sur le *Scleroderma*, remarques que je rapporte intégralement, étant donné leur intérêt et leur analogie avec celles faites par moi-même. "Mr. Haliday, however took several specimens of both sexes of a *Scleroderma* in a chamber where *Attagenus pellio* abounded in the mattresses stuffed with the husks of Indian Corn; and on another occasion he found a swarm of the former between the sheets of a bed upon the sofa of a house at Lucca, probably stuffed, as he conceived, with hair or wool infested by the latter".

Après les indications de Saunders il faut arriver jusqu'au travail de Schulze (1919) pour avoir d'autres informations étendues et précises. Cet auteur fait remarquer la présence en Macédoine de *Scleroderma* qu'on retrouve assez communes dans les maisons et qui piquent l'homme. Il ajoute avoir lui-même subi la piqûre du *Scleroderma sydneyanus* Westwood, avec des conséquences peu remarquables, le tout se réduisit à une légère réaction cutanée de faible durée. La population cependant, à ce que dit cet auteur, avait une peur exagérée de ces microhyménoptères ce qui fait penser que les effets de la piqûre pouvaient être parfois assez remarquables. Après 1919, si l'on fait exception pour la citation sur le Congo faite par Bequaert (1924), la littérature se trouve toute groupée dans ces dernières dix années.

En 1948 Bernard et Jacquemin annoncent quelques cas de piqûre de *Scleroderma abdominalis* Westwood parmi la population d'Alger (mai 1948), piqûres presque toujours suivies par des réactions cutanées d'une certaine intensité et d'une durée assez longue, accompagnées souvent par des phénomènes d'anaphilaxie assez sérieux (vertiges, nausées,



troubles généraux). Deux ans après Mandoul, Bernard et Jacquemin (1950) reprennent la question et résument les observations de piqûres de la part du *Scleroderma abdominalis* faites par eux-mêmes à Alger (avril, mai 1948; juin, août 1949). Le cas clinique varie peu d'un cas à l'autre. Harrant et Huttel (1950) parlent d'accidents qui se vérifient à Montpellier (France) à cause de la piqûre du *Scleroderma domesticum*, espèce que Bernard et Jacquemin (1948 page 165) considéraient comme peu nuisible pour l'homme.

Diss et Timon-David (1951) toujours se rapportant au *Scleroderma domesticum* font remarquer des cas de piqûres étudiés par eux-mêmes à Marseille. Ils disent avoir étudié sur un individu des phénomènes généraux assez sérieux "état fébrile jusqu'à 39°5, courbatures, anorexies, adenopathies, et, au niveau du scrotum, oedema important. . ." Theo-

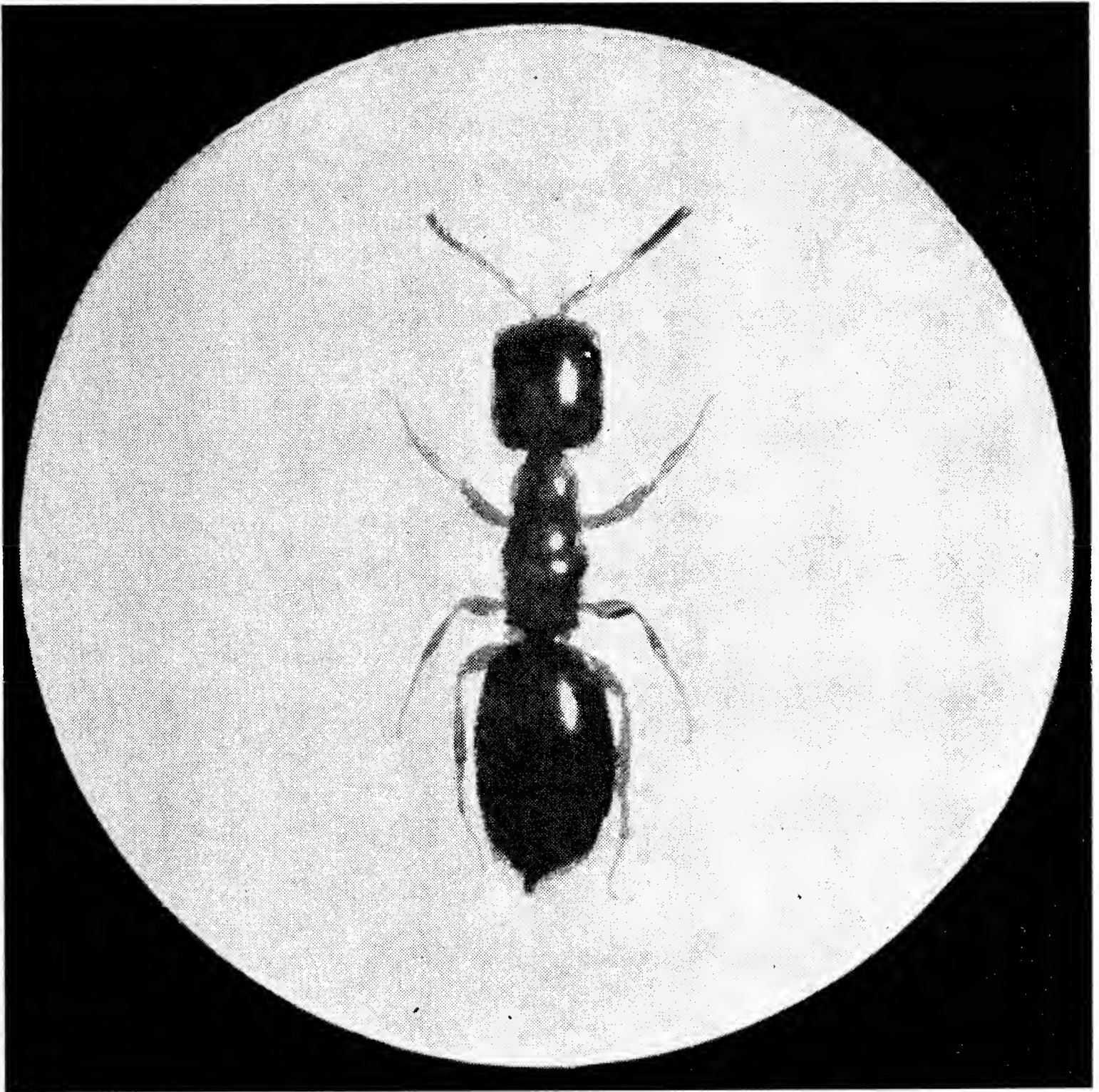


Fig. 1. *Cephalonomia benoiti*, femelle.

dorides (1951) fait connaître un cas personnel de piqûre à un doigt dans le Laboratoire Arago de Banyuls (3/VI/1950). Il dit avoir senti sur le moment une douleur aiguë, suivie peu après par l'apparition d'une papule rouge qui dans l'espace de quelques heures s'en alla sans laisser d'autres inconvénients, ce qui l'amenerait à penser que la piqûre du *Scleroderma domesticum* soit moins douloureuse et avec une réaction cutanée moins persistante par rapport au *Scleroderma abdominalis*. Jacquemin et Vayssière (1950) illustrent et font remarquer un premier cas de piqûre dans l'Afrique du Nord de la part du *S. domesticum*.

Le 27 mai 1950 l'un d'eux fut piqué d'abord sur le côté droit du cou, et ensuite à la hauteur de l'articulation sterno-claviculaire; l'impression de la piqûre fut comparée à celle d'un liquide brûlant qui pénètre dans la peau. Les phénomènes qui suivirent n'étaient pas différents, en ligne générale, de ceux déjà décrits pour le *S. abdominalis* (apparition de

papules du diamètre de 5 mm., érythème tout autour, oedème étendu à toute la région latérale du cou avec par conséquent une mialgie), phénomènes qui après quelques jours commencèrent à s'en aller, exception faite pour les papules qui persistèrent pour une semaine. Mariani (1952) qui le premier en Italie s'est arrêté pour observer les *Scleroderma* par rapport à l'homme, dit qu'à Naples et à Palerme, les cas de piqûres de la part du *S. domesticum* sont très communs. Il dit en effet que presque tout le monde interrogé à cet égard, se rappela avoir été piqué par une fourmi brune très petite et très agile, et, bien que les versions sur les conséquences de la piqûre aient de légères variantes, tout le monde était d'accord pour déclarer que la piqûre donnait la sensation d'une épingle brûlante qui pénètre dans la peau. Certains disaient avoir senti seulement une douleur très forte qui ne dura que quelques minutes, d'autres se rappelaient que la piqûre leur avait laissé des papules remarquables et persistantes, et d'autres encore que cela avait été la cause d'une dermatose du type allergique.

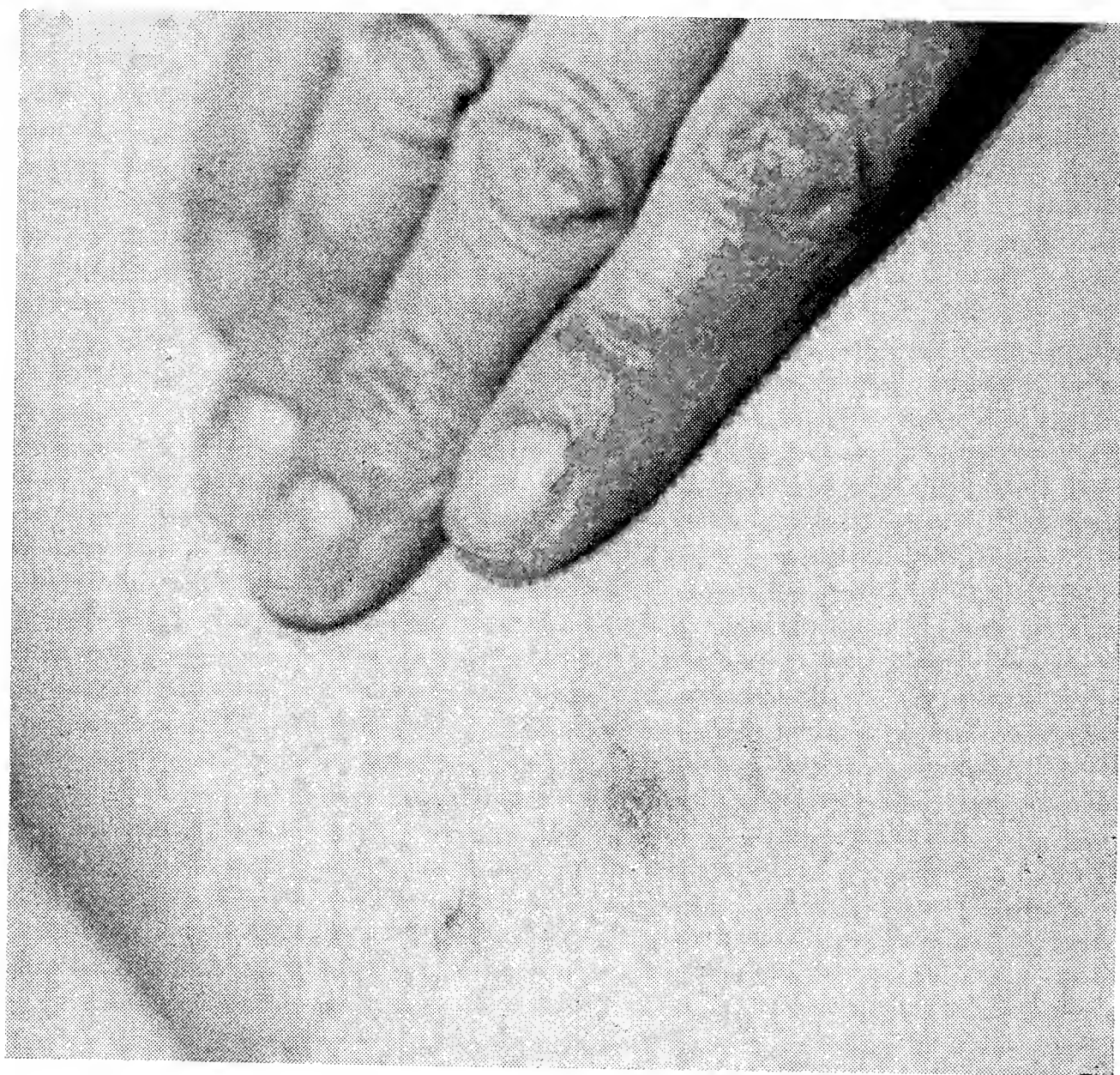


Fig. 2. Les effets de la piqûre de *Cephalonomia* (lésion sur les cuisses datait d'une dizaine de jours).

Mercadel Peyri (1953) est le premier en Espagne qui parle amplement du *S. domesticum* du côté médical. Le cas clinique des différents cas de piqûre signalés par cet Auteur ne diffère pas, en ligne générale, de celui déjà illustré par d'autres Auteurs; il ne parle pas de cas avec troubles généraux d'une certaine importance. Vermeil (1954) nous fait connaître le cas d'une piqûre à Tunis faite par le *S. domesticum*. Jacquemin et Marchetti racontent que les derniers jours d'avril 1953, une dame en cherchant de vieux meubles vermoulus, fut à plusieurs reprises piquée par les *S. abdominalis* sur une surface plutôt réduite. En ce cas aussi la piqûre, très douloureuse, fut suivie par des papules endurcies larges d'un centimètre, rouges et fort prurigineuses, qui, dans l'espace de 6/7 jours furent remplacées par des tâches jaunâtres, qui lentement disparurent en s'écaillant comme des pellicules. On ne vérifia aucun cas d'anaphylaxie à caractère général. Jacquemin et Arles (1955) ont établi pour la première fois que même le *S. unicolor* Westwood est nuisible à l'homme dans l'Afrique du Nord. Le cas se rapporte à un enfant de quatre ans qui dans la première quinzaine de juin 1952, fut piqué, à deux reprises sur des différentes parties du corps:



bras, cuisses et fesse droite, avec une remarquable irritation cutanée et des manifestations semblables à celles déjà décrites pour le *S. abdominalis*.

Des auteurs pensent cependant que le venin de l'*unicolor* est moins actif que celui de l'*abdominalis*, en effet, la petite victime malgré les nombreuses piqûres ne ressentit aucun trouble général. D'après cette rapide revue des différents cas de piqûre cités dans la littérature, on déduit que les substances qui font part du venin des *Sclérodermines* ne devraient pas différer, en ligne générale de celles qui constituent le venin des autres hyménoptères aculéates, dont à plusieurs reprises s'est occupé M. Leclercq (1951).

Le choc par anaphylaxie, produit sans doute de l'istamine, à laquelle on doit la vasodilatation locale et l'enflure qui en suit. Ainsi que pour tous les hyménoptères aculéates en général, même pour les *Sclérodermines* les conséquences de la piqûre sur l'organisme sont en rapport, naturellement, avec la région où la piqûre a été pratiquée; en correspondance d'un nerf elle sera plus douloureuse et ses effets dureront plus longtemps. Si l'aiguillon pénètre au contraire dans une veine superficielle les symptômes locaux seront presque nuls, tandis que les troubles généraux seront rapides et sérieux.

Ces piqûres présentent quelque fois l'avantage d'éliminer aussitôt le venin par la voie urinaire, et de ne pas laisser des suites remarquables. Les phénomènes plus ou moins graves d'intoxication sont liés, comme nous avons vu pour le cas de M. et Mme B., à la réaction spécifique de chaque individu.

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# Présentation du "Bulletin Signalétique d'Entomologie Médicale et Vétérinaire"

Par J. L. HOUPEAU

Office de la Recherche Scientifique et Technique Outre-Mer  
Paris, France

*Ce Bulletin paraît tous les mois.* Chaque numéro comprend deux fascicules. Le *premier fascicule* donne les titres des articles et des ouvrages avec indication du nombre des figures, des cartes et des références bibliographiques. Quand un article comporte des résumés en plusieurs langues, chacun d'entre eux est indiqué. Chaque référence comporte le sigle de la bibliothèque scientifique de Paris où le périodique a été consulté.

Des précisions sont ajoutées à certaines références quand il y a lieu; ce sont essentiellement:—le nom des espèces nouvelles décrites; le nom des espèces étudiées dans le travail cité; des précisions géographiques. Ce premier fascicule comprend cinq tables:

1. Table alphabétique des publications périodiques et des ouvrages analysés. En 1955, 500 périodiques ont été consultés, fournissant près de 2,500 références.
2. Table des Congrès, Colloques, Symposium, etc.
3. Table alphabétique des auteurs.
4. Table méthodique des matières.
5. Table géographique.

Le *deuxième fascicule* présente les références de façon à pouvoir les découper et coller sur fiches 75/125 mm.

Le Bulletin signalétique d'Entomologie médicale et vétérinaire est rédigé par le Dr J. L. Houpeau et édité par l'Office de la Recherche Scientifique et Technique Outre-Mer, 20 Rue Monsieur, Paris (7ème). Le prix de l'abonnement pour 1958 est de 3.000 Frs. pour la Métropole et l'Union française et de 3.500 Frs. pour l'Etranger. Le Bulletin signalétique peut également être obtenu par échange.



# Les Sixièmes Congrès Internationaux de Médecine Tropicale et du Paludisme

Par J. FRAGA DE AZEVEDO

Instituto de Medicina Tropical

Lisbon, Portugal

Avec la réalisation du X Congrès International d'Entomologie qui se termine aujourd'hui nous venons d'assister à une des plus importantes manifestations culturelles et à une démonstration très élevée de collaboration de plus de 60 pays dans le domaine de la science.

On peut dire en réalité que presque tous les sujets d'intérêt pour l'Homme concernant les arthropodes, et nous ne devons pas oublier que ce groupe est le plus riche de la Zoologie, ont été traités, étudiés et discutés et sur beaucoup d'entre eux des résolutions précises ont été prises.

En ce qui concerne plus particulièrement notre section, l'Entomologie Médicale et Vétérinaire, nous avons assisté à la présentation et discussion de problèmes importants de morphologie, d'écologie et de biologie des espèces entomologiques comme agents de maladies ou comme vecteurs d'agents pathogènes pour l'Homme et pour les animaux. De cette façon une contribution remarquable pour le progrès de cette science a été apporté.

D'ici 4 ans aura lieu de nouveau un Congrès International d'Entomologie. Il serait souhaitable d'y revoir tous ceux qui sont ici présents. Cependant en 1958 auront lieu à Lisbonne les 6<sup>èmes</sup> Congrès Internationaux de Médecine Tropicale et du Paludisme. Comme Président de la Commission Internationale Intérimaire chargé de l'organisation de ce Congrès et comme Président de la Commission Nationale de mon pays qui doit les recevoir je m'adresse à vous pour vous demander de nous apporter toute votre précieuse collaboration pour cette réunion.

On sait bien que le progrès réalisé par l'Homme sur de vastes régions des tropiques a été le résultat surtout du progrès de l'Entomologie Médicale. En réalité comment aurions pu nous protéger contre le paludisme, la fièvre jaune et autant d'autres maladies endémiques transmises par les arthropodes sans les très brillantes conquêtes de cette science? Au delà, cependant, des grands progrès déjà réalisés dans ce domaine il y a encore beaucoup de problèmes qui attendent une solution. Ainsi, encore aujourd'hui les glossines ne permettent pas l'occupation totale de grands et riches territoires, étant donné que nous ne disposons pas aujourd'hui de moyens pratiques de lutte contre ces insectes de vastes régions de l'Afrique. Pour certaines formes de filaries, comme la *W. bancrofti* et la *L. Loa* nous ne disposons pas aussi de moyens efficaces de lutte contre leurs vecteurs. Il y a encore dans les tropiques quelques maladies à virus peu connues en ce qui concerne aussi leurs agents vecteurs. Le nombre d'*Anopheles* vecteurs du paludisme insecticide-résistants augmente de jour à jour. Et l'on pourrait citer beaucoup d'autres exemples pour rappeler l'importance des études d'Entomologie Médicale pour les pays tropicaux. De cette façon les prochains Congrès Internationaux de Médecine Tropicale et du Paludisme qui se tiendront au Portugal auront besoin pour leur succès de la collaboration active et de l'intérêt des spécialistes en Entomologie Médicale.

Certainement tous les pays ici représentés seront invités à collaborer à cette importante manifestation de culture et de solidarité humaine, comme sont d'ailleurs tous les Congrès Scientifiques Internationaux. Cependant j'ai considéré utile, en profitant de l'opportunité de cette réunion, où sont présents certains des plus distingués entomologistes médicaux du Monde de m'adresser à vous pour qu'à partir de cette date vous puissiez inscrire dans vos programmes de travaux des problèmes d'entomologie qu'intéressent plus particulièrement les pays tropicaux.

On trouve ici des participants provenant de toutes les latitudes, mais quelque soit la latitude tous les travaux dans le domaine de l'Entomologie ont la plus grande importance pour l'Entomologie Médicale des régions tropicales. Ainsi, par ex., lorsque le Canada ou les Etats-Unis de l'Amérique du Nord étudient les simuliidés ou la transmission des virus

des encephalites par les arthropodes, ils apportent une remarquable contribution pour la connaissance des sujets similaires des régions tropicales. Cela sans parler de l'intérêt général de quelques études comme l'étude des insecticides, des molluscocides par ex., et de beaucoup d'autres.

De cette façon je me permets d'espérer votre importante collaboration et dans ce sens je formule mes meilleurs voeux pour la présence de tous ici présents dans les prochains Congrès Internationaux de Médecine Tropicale et du Paludisme à Lisbonne en 1958.



# AUTHOR INDEX FOR VOLUME 3<sup>1</sup>

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